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PHOTOCHEMICAL OXIDATION OF BUTTER

by

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ABSTRACT

Samples of butter taken at random from grocery stores were subjected to different tests to determine whether the fluorescent light used in the display cases had an oxidizing effect on the butter. Fresh butter samples were exposed to fluorescent light in the laboratory. The state of oxidation after various exposure times was determined by chemical tests and by organoleptic evaluation. The butter samples were exposed at three different distances from the light source. Unwrapped butter and butterfat samples were also exposed to the light. This was done to find out whether the phospholipid fraction of the fat has any effect on the oxidative deterioration of the butter under the light.

The changes in peroxide values and carbonyl values indicate that fluorescent light had a prooxidant effect on the butter. The organoleptic tests correlated with the results of the chemical tests. The butter was also bleached as a result of the light exposure, indicating that the oxidation degraded the carotenes.

The harmful effects of fluorescent lighting in the grocery stores were indicated by the results of the tests performed on the samples taken from the supermarkets.

The use of better packaging materials that would protect the butter against light seems an urgent requirement.

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INTRODUCTION

The autoxidation of edible oils adversely affects the flavour quality and the vitamin and essential fatty acid contents. The ingestion of oxidized fats also has toxic effects.

For these reasons, the study of oxidative deterioration of edible oils or fats has been the subject of numerous investigations. Ways and means for evaluating the keeping quality and incipient rancidity of fats have given rise to the development of various methods of accelerated tests. These methods were primarily used for evaluating the resistance of fat to oxidation and also for evaluating the effectiveness of antioxidants. The general characteristics of these tests are that fats and oils are subjected to accelerated conditions of oxidation under carefully controlled environmental factors.

The changes occuring during the oxidation of fats are characterized by an induction period which is followed by rapid oxidation. The factors that speed up oxidation are metals, light, oxygen, moisture and high temperatures. The presence of natural and added antioxidants alters the relationship between the pro-oxidants and the lipid system. The study of oxidative deterioration of a given lipid system is complex because of the various factors enumerated above which often operate simultaneously.

Studies on the oxidative deterioration of fats and oils under normal conditions of storage have shown that there is still room for improvement in so far as the keeping quality of foods are concerned. Recent investigations (43, 44, 127, 134) concern the effect of artificial lighting in retail stores on lipid containing foods. This arises from the fact that supermarkets are always intensely lighted and foods are sometimes no more than a few inches away from the source of light while on the display shelf.

The purpose of this study was to find the effects of fluorescent light on butter and the butterfat as shown by the results of chemical and organoleptic tests.

REVIEW OF LITERATURE

Factors Affecting Oxidation

Metal - In 1917, Hunziker and Hosman (72) found that copper as well as iron catalyzed the development of tallowy flavour in butter. Either an iron nail or a copper wire imbedded in the butter caused bleaching and tallowiness. The addition of colloidal hydroxides of iron and copper in minute quantities to butter (4 drops per 180 grams of butter) was tried with the result that the copper-contaminated sample became tallowy in 8 days, whereas the iron-contaminated sample did not show this defect after two months in storage at 32°F. Pure butterfat emulsified with casein and a slight excess of alkali showed slight tallowiness and bleaching after 5 days at room temperature in the presence of iron, copper and brass. These samples were intensely tallowy and bleached after 28 days. Samples containing nickle and tin, which were normal after 5 days, had turned slightly tallowy without bleaching, and the butterfat of normal lactose content emulsified without acid, alkali or metal addition remained normal. The iron-contaminated sample containing alkali became fishy. It was observed further by these workers that in all cases of tallowiness the defects appeared first at the surface of the butter and then inward.

In 1922, Emery and Henley (35) found that copper and iron containers caused oxidative defects in lard, corn oil and cottonseed oil. They recommended lacquering as a preventive measure. Thome

and Olsson (148) also mentioned the prooxidant effect of copper in a pump used to prepare butter. This is because small amounts of copper pass into solution when the pump was used to circulate the cream. Koops (79) also reported that oxidative deterioration of butter from cultured cream during cold storage is strongly promoted by copper contamination. He recommended the use of antioxidants in addition to copper elimination.

Several factors influence the dissolution of the metal of containers. Whitfield (158) observed that greater corrosion occurred at 144°F than at 60°F. Increased solubility of copper up to158°F was observed (47).

Copper containers with oxidized surfaces showed increased corrosion (118). The presence of oxygen has been shown to increase the rate of copper corrosion. However, the presence of carbon dioxide decreases it (46, 47, 48, 94, 118).

Since off-flavours are believed to be due to oxidation, the factors affecting the oxidation of butterfat received increasing attention. Briggs (8, 9) showed that certain metallic catalysts hasten the oxidative reaction. Parodi (105) studied the types of butter boxes and cartons and the chemical nature of the vegetable parchment wrapping. It was found that when the copper content of the parchment is kept below 5 ppm there was no evidence of surface oxidation after storage at 10°F for 6 months. With increasing amounts of copper, surface grades deteriorated. The effect of metals

on washed cream was also studied (144). It was observed that flavour defects were fishy and metallic. Tarassuk _et al_. (145) suggested that the possible origin of trainy flavour in the fat globule membrane is produced by the oxidation of the unsaturated fatty acids of the phosphatides and is accelerated by metals, increased temperature and pH. They indicated that if the membrane protein-bound copper is removed by a copper binding antioxidant no oxidation will occur.

Milk and milk products have natural copper as part of their constituents. Several investigators have shown that copper combines with proteins. It is their belief, however, that the combination is a matter of adsorption rather than direct chemical combination (102, 149, 150).

Because of the importance of copper in milk, a test for copper was developed (121) based on the peroxidase reaction. The reagents used are p-phenylenediamine, alpha naphtol and hydrogen peroxide. When these are added to freshly pasteurized milk or cream, the rapidity of formation of a blue color is proportional to peroxidase content and since copper acts as a catalyst in the reaction, the test can be used as the measure of copper content. This test was modified by Turgeon _et al_. (152) who also found that vitamin C complicated this application. Later, Herrington and Brereton (68) developed a flame test for determining the presence of copper in metals or alloys. A polarographic determination of copper and iron in fats was developed by deMan and Engelhardt (30).

Air and Oxygen - In 1910 Nestrelayev (97) observed the difference in susceptibility to oxidation by air and light of different butter samples. He learned that the larger the content of unsaturated acid present the greater the effect of light.

Browne (12) studied the decomposition of butterfat and found a marked increase in free and volatile acids and a considerable decrease in the content of insoluble acids. He reasoned that disintegration of the fats was primarily due to the action of active oxygen, one atom of which was liberated for each atom absorbed at the points of unsaturation. Greenbank (52) found that butterfat did not undergo oxidation when free fatty acids were removed by steam distillation and when stored under vacuum in diffused light for three years. He found that the oxidation of fats in the presence of heat and air, and in the absence of light, reduced the induction period after three months of storage from 248 minutes to 41 minutes.

Greenbank (51) suggested packing butter in evacuated containers and then releasing the vacuum by inert gas to prevent oxidative deterioration. Prucha *et al*. (113) reported that storage of butter in an atmosphere of carbon dioxide in tightly sealed cans improved its keeping quality.

Sommer (136) found that presence of air caused fishiness in butter. Overworking of butter resulted in increased air content and therefore increased oxygen available for the oxidative deterioration.

Giermak (49) stated that a smoothly finished surface permits

good contact between the butter and wrapper which aids against surfact taints, off-flavour, mold development and oxidation. He believed that uneven surface results in the failure of the treated liners to adhere to the butter surface thus permitting air pockets to form which may cause development of defects. Columbic _et al_. (23) examined the flavours produced during light reversion of soybean oil under atmospheres of different oxygen concentrations. No decrease in the tendency to revert under different conditions was observed.

Light - Early workers (8, 11, 35, 38, 52, 65, 123, 124, 126, 131) have established the important accelerating effect of light on fat oxidation. Nestrelayev (97) found that butter from different parts of the country differed in their susceptibility to oxidation when exposed to air and light depending on the content of unsaturated acids. de Vleeschauwer _et al_. (154) found that the oxidation of ascorbic acid in both water and milk was increased by the presence of riboflavin and light. The estimation of steam volatile monocarbonyl compounds from oleate, linoleate and linoleate esters was carried out by Gaddis _et al_. (45) after exposing the esters to ultraviolet light. Pedersen and Andersen (109) found that during storage of ascorbic acid-enriched milk in darkness, the ascorbic acid content decreased slowly and no off-flavour could be detected after 2 days of storage. On exposure to daylight, the ascorbic acid was decomposed rapidly and a marked tallowy flavour was produced. In a strong daylight noticeable changes took place when the milk was exposed for only 15 minutes. Radema (115) found that skim milk is most sensitive to light-induced oxidation.

Different studies on the autoxidation of fats show that shorter wavelengths are mainly responsible for the initiation of the chemical reaction. In 1932, Coe and LeClerc (20) reported that ultraviolet light is more effective in promoting oxidation than sunlight. Greenbank and Holmes (55) reported that the lower wavelengths are more effective in their study of different oils. In 1947 McConnell and Eoselen (92) also reported on the more effective action of the lower wavelengths. Soldberg and Reidar (135) found that off-flavour in milk is caused by light of wavelengths below 500 millimicrons. Brown glass bottles, although providing good protection are not completely effective in preventing the detrimental effect of exposure to light.

Fifteen years later a study of the effect of different wavelengths present in fluorescent light was made by Scott (127) who concluded that the wavelengths in the ultraviolet portion of the spectrum had a definite effect on the quality of butter. His evaluations were based on organoleptic tests. In 1965 Moser *et al*. (95) published a paper describing the development of off-flavours in oils and other food products while on the shelves when exposed to fluorescent light. They devised an accelerated test apparatus wherein the oils are exposed to fluorescent light and the samples tested at regular intervals.

Malm and Hildingston (91) made a study of the effect of lighting in cold storage cabinets in retail stores. The yellow light from filament lamps and the blue-white and warm light from tubular

lamps were equally active. An exposure of even one hour to any of these sources of light caused a rise in peroxide number. They found that an aluminum foil wrapper was effective protection against the effect of light. Futschik and Aigner (43) made a similar study. They exposed prints of butter wrapped in aluminum foil, parchment and unwrapped. They used a filament lamp of 40 watts or an ultra-violet lamp of 30 watts for 24 hours at a temperature of 15°C. Taste, smell and appearance of butters wrapped in aluminum foil were satisfactory irrespective of the type of light, but unwrapped samples and those wrapped in parchment showed a marked deterioration in quality.

Futschik and Aigner (44) studied the effect of light on the quality of milk, whipping cream, butter and cheese. Thirteen types of fluorescent tubes made by 4 manufacturers and giving white and yellow light (100 - 260 lux) were compared at a distance of 1.5 meters from the products enclosed in a cabinet at 10° - 12°C; the controls were kept in darkness under similar conditions. The changes in the quality of the product were assessed organoleptically after 24 hours and 72 hours illumination. They found that with the exception of Edam cheese the illumination of unwrapped products gave rise to the development of off-flavours, the intensity of which depended on the product and the colour of the light. White light was found to be more harmful than yellow light. Milk in clear glass bottles became defective after 24 hours while those in brown bottles remained unchanged irrespective of the colour of the light. Whipping cream was more sensitive to yellow light than to white light. With butter white light was found to be most harmful. Aluminum foil was found to

protect the butter from deterioration whereas parchment paper was ineffective. The deterioration was most pronounced in unwrapped butter. Edam Cheese was hardly affected by fluorescent light.

Moisture - Fierz-David (38) believed that rancidity was produced by air, light and water; that fats containing saturated fatty acids were oxidized to their corresponding methyl alkyl ketones and that unsaturated fatty acids were split into aldehydes and acids. Holm (69) however, found that the presence of water in fat extended the induction period. Later Greenbank and Holm (53) expressed the same belief when they made a study of the factors concerned in the autoxidation of butterfat. They found that the presence of water or water vapor seems to retard the autoxidation of fats. Ritter and Nüssbaumer (123) explained these results on the basis that butter serum must contain some substances which retard the oxidation of the butterfat. They found that the rate of oxidation of pure butterfat was scarcely affected by the addition of a quantity of water equal to that contained in butter. It appears that in butter the moisture itself does not play as important a role as does the material which it carries. They explained this effect on the basis of the cephalin and lecithin content of the serum. Thome (196) also believed that since the moisture content of butter is always high, changes in it will have little effect on the oxidative deterioration of butter.

Radema (114) examined samples of spray-dried whole milk from five factories. Portions of each sample were adjusted to moisture contents of 1.7, 2.4 and 4.2%, each portion being further subdivided

and stored in the dark at temperatures of 9, 20, 30 and 37°C for about one year. Samples were examined at intervals for flavour, moisture content, sulfhydryl reaction, peroxide numbers, reducing properties, TBA test and color in ultraviolet light. The results showed that the keeping quality of spray-dried whole milk decreased with increasing storage temperature and that high moisture content encouraged the development of tallowy flavour while a low moisture content encouraged a "glue-like" flavour.

Titov (151) reported that Meleshin butter contains moisture in a more highly dispersed state than ordinary churn butter and he believed that this particular property was responsible for the better keeping quality of the butter. Bognanov and Titov (10) also studied the effect of moisture dispersion on the keeping quality of the butter. The role of protective substances said to be contained in the butter plasma was investigated by comparing butters made from two lots of "cream" prepared from butter by the addition of (1) salted water or (2) milk. When examined after 1 year's storage at -10°C the butterfat extracted from the latter butter had a better flavour and received a higher score than that from the former. In further experiments normal unwashed butter with finely dispersed moisture was awarded more points after 1 year's storage at -10°C than that containing moisture in large droplets. Csizar (25) attempted to show a relationship between water, fat and protein content of butter and its keeping quality. There was no correlation between acidity or air-content of butter and its keeping quality but the evenness of moisture distribution affected the keeping quality.

Salt - Washburn and Dahlberg (156) showed that salt, aside from its antiseptic property, hastened the deterioration of butter. Oily, metallic and fishy flavours showed up to a greater extent in salted than in unsalted butter. Guthrie *et al*. (60) showed that salt in butter greatly retards bacterial growth. Butters containing no salt were prone to become old or stale, but seldom oily. They concluded that this may be attributed to inhibited bacterial growth since they were stored at 10°C.

Jacobsen (79) reported that the destructive action of salt apparently was of greater importance than freezing in reducing the number of bacteria in salted butter. Thurston and Brown (150) concluded that since the concentration of salt is associated with the development of fishy flavour, and since fishy flavour apparently is the result of hydrolysis and oxidation of lecithin, salt tends to favor oxidative changes. Washburn and Dahlberg (155) have found that salted butter is more likely to turn fishy in storage than unsalted butter. Sommer and Smit (137) also reported that salt caused fishiness in butter.

Pandit *et al*. (104) compared butter samples with no or up to 3 washings, with 0 - 2.5% sodium chloride and from churned cream with acidity adjusted to 0.22 - 0.75% lactic acid. Acid values and peroxide values were measured and flavour was assessed at 7-day intervals during storage at 40°F. It was found that unwashed unripened butter containing 2% sodium chloride had the best keeping quality. Its flavour was improved at 0.45% acidity and it could still be stored

for 5 weeks. Kratochvil et al. (81) studied the keeping quality of unsalted and salted butter (1.5% salt) made in a wooden butter churn from cream ripened to 11.9° SH. The butter was wrapped in parchment paper or lined aluminum foil and was stored at 4 or 18°C for 48 days and tested organoleptically and chemically. The results showed that the salting had an adverse effect on organoleptic quality of butter during storage, particularly at the higher temperature and when the butter in parchment paper was exposed to light and sunshine. The use of aluminum foil improved substantially the keeping quality of salted butter.

Malm (90) found that high salt content (about 2%) retarded bacterial development but tended to promote oxidative deterioration. Grishchenko et al. (58) obtained evidence that the tendency of butter to become oily increased with increasing salt content and this became more pronounced the longer the butter was held in storage. Barnicoat (4) pointed out that deterioration of butter during storage at 14°C made from good quality cream was increased by high acidity, high copper and iron content and high salt content. Anderson and Olson (2) noted a slight deterioration of butter samples which they stored for 7 days at 80°F especially those with salt concentrations of 12 to 14% in the non-fat portion. Loftus-Hills and Conochie (87) found that a combination of pure magnesium chloride and sodium chloride in butterfat promoted oxidative deterioration.

Acid - Many research workers (8, 61, 88, 103) have noted the detrimental effect of ripening cream on the keeping quality of the butter. Mortenson

(96) found that the percent acid in the cream was inversely proportional to the keeping quality. Sommer and Smit (137) found that the rate of oxidation is proportional to acidity.

Ritter (122) found that acidity was an important factor in the development of fishy flavour. Holm (69) correlated the change of butter flavour in storage with chemical changes which were brought about by oxidation. Greenbank (51) showed the importance of the use of high quality sweet cream for churning butter. He reported that cream having an acidity of 0.13 to 0.19% results in a butter with better keeping quality than with higher acidity. He found that butter made from cream of less than 0.2% acidity never became fishy. Storgards and Hietaranta (138) however, argued that the trimethylamine arising from lecithin and suspected to be responsible for off-flavour quality of butter is not considered an oxidation product since it is formed as a result of the reduction of lecithin. They argued that by increasing the pH decomposition of lecithin is prevented.

Masek _et al_. (92) confirmed the adverse effect of high acidity on butter stability. Rahmn (116) recommended that the pH of print butter intended for frozen storage should be between 6.4 and 7.1 in order to obtain the best keeping quality.

Dixon (31) reported that the pH of dairy products is temperature dependent. He recommended that for critical work temperature control to within plus or minus 1°C is required to equal the accuracy of pH measurement. Pont (110) observed a better keeping quality of

butter made from cream of lower acidity. Thurston and Brown (151) concluded that acidity appears to play an important role in the deterioration of butter which, under some conditions, appears to be the result of oxidation.

Antioxidant - Greenbank and Holms (56) found hydroquinone, phthalic acid and maleic acid to be good antioxidants. Of these three, maleic acid was the best. Olcott and Mathill (101) found pyrogallol, hydroquinone, pyrocathecol and hydroxyhydroquinone to be excellent antioxidants. Coe and LeClerc (21) found that maleic and phtalic acid, hydroquinone and pyrocathecol act as antioxidants.

Olcott and Emerson (100) found tocopherols to have antioxidant properties in lard. Takashi and Yoschiichi (142) found the phenols in incompletely burned oak smoke to have antioxygenic properties. Ritter and Nussbaumer (124) reported antioxidative properties of hydroquinone.

Koenig (78) reported that parchment paper used as butter wrapper treated with oat flour hexane extract, which they termed Avenol, had protective action against the development of rancidity. Dahle and Josephson (27) found that by treating parchment paper with finely ground oat flour which they called Avenex, flavour defects were retarded at 45°F but had little benefit as long as butter remained in storage at -15°F. They found that a water extract of Avenex improved the keeping quality of butter without affecting the flavour or incorporating sediment into the butter. Dahle (26) found that oat flour in

butter delayed stale and cheesy flavours. Several workers (24, 26, 27 78) found a similar retarding effect on the development of oxidized flavours in butter. They also found that the use of avenized parchment paper retarded surface flavours and improved keeping quality.

El-Negoumy and Hammond (34) studied the effect of different antioxidants in butter stored at temperatures from -18 to 38°F. They found that development of oxidized flavour was not significantly retarded by antioxidants at either temperature. Kotova (80) studied the effectiveness of several antioxidants in delaying fat oxidation as well as their effect on organoleptic properties of butter. The antioxidants were propyll gallate, ascorbyl stearate, norhydroguiaretic acid and carotene. The antioxidants were added to high fat cream and the keeping quality of the resultant butter presumably made by the Meleshin process was assessed from the increase in peroxide value of butterfat at 10°C during 48 hours. The results show that propyl gallate, propyl gallate plus carotene and nordihydroguairetic acid plus carotene very effectively prevented the increase in peroxide value, ascorbyl stearate was less effective and carotene was ineffective. The taste of the butter was found to be affected to a varying extent by most of the antioxidants tested.

Lea(84) observed that the relative efficiencies of a series of antioxidants can be altered considerably by comparatively small changes in the composition of the subtrate or even the temperature of oxidation and that the change in activity from one complex food to another can be very large.

Techniques in Autoxidation Studies.

A variety of techniques are employed in autoxidation studies, some of these are highly specialized, such as for example countercurrent extraction, low temperature crystallization, molecular distillation, chromatography and urea complex formation.

Molecular distillation (141), chromatography (37) and low temperature fractional crystallization (1, 141) were used in earlier attempts to isolate methyl oleate peroxides. Urea complex formation (22, 128) was used later to isolate the peroxides by precipitating the nonperoxide portions of the autoxidation mixtures. Solvent extraction (112), countercurrent extraction (42, 77), and reversed phase partition chromatography (128, 139) were made use of in obtaining peroxide concentrates.

Secondary autoxidation products have been steam-distilled from milk fat (66) and from autoxidized methyl isolinolenate (62) and collected in a dry ice trap during the autoxidation of methyl linolenate (74). These volatile fractions have further been characterized by chromatographing the carbonyl components as the respective 2, 4 dinitrophenylhydrazone derivatives (16, 62, 74). Isolation of secondary oxidation products was also done by Lundberg and Chipault (89) in their study of oxidation of methyl linoleate at various temperatures.

It is realized that many common analytical methods may yield false values when applied to highly oxidized oils because of

the interference of large amounts of peroxides.

Review of Methods for Determination of Autoxidation Products.

Peroxides.

Iodimetry - There are several modifications of this method. They are all based on the assumption that potassium iodide and hydroiodic acid, when brought in contact with fatty peroxides, liberate iodine quantitatively in some simple stoichiometric manner, with two atoms of liberated iodine equivalent to 1 atom of active oxygen.

The peroxide method of Wheeler (156) is most widely used. Improvements on this method were discussed in a recent study where exclusion of oxygen and light from the reagents and reaction flasks were suggested (119).

Polarography - Lewis *et al*. (85, 86) found a linear relationship between wave height and peroxide value in the early stages of the oxidation of fats. Ricciuti *et al*. (117) made a study of the polarographic behaviour in a more detailed investigation. They showed that peroxides, hydroperoxides, aldehydes, ketones conjugated with a double bond and alpha diketones could be measured polarographically. They demonstrated that hydroperoxides could be determined quantitatively in the presence of other peroxide types. Later Kuta and Quackenbush (82) published similar results.

Ferric thiocyanate method - This is a colorimetric method. The main criticism of this procedure is that it is subject to considerable

error. Several workers (17, 18) have described such a method but the peroxide value as determined by this means is higher than that usually found with the iodometric method.

Dichlorophenol-indophenol method - This method was introduced by Hartman and Glavind (63). It also yields high values in the presence of air (64). The values, however, are reproducible and are useful in comparative studies.

Carbonyl Compounds

It is a well-established fact that the major constituents of oxidized flavour of dairy products are carbonyl compounds (39, 40, 75, 76). For this reason several methods for the estimation of these compounds have resulted from many studies. Prill (111) devised a method for estimation of the alpha diketo compound. It is based on oximation of the diketo compounds present in the fat. The dioximes which are formed by the reaction are converted to derivatives of iron, nickel or copper, which are soluble in benzene with the formation of characteristically colored solutions.

O'Daniel and Parsons (99) suggested that the color developed during fat saponification may serve to indicate the presence of carbonyl compounds. They believed that the color developed during such treatment is due to the presence of quinoid compounds which results from aldol condensation of alpha diketones.

Lappin and Clark (83) developed a sensitive method for the estimation of carbonyl compounds using 2, 4-dinitrophenylhydrazine.

This was used by Neumer and Dugan (98) for estimating the stability of dry dog food and showed that the break in the rate curve of development of carbonyl compounds occurred simultaneously with the change in the rate of development of peroxides, The method of Lappin and Clark was modified by Berry and McKerrigan (6). They made use of two different wavelengths in the estimation of the presence of saturated and unsaturated carbonyl compounds. Henick _et al_. (67) further modified this procedure by using carbonyl-free benzene to minimize error. Trichloracetic acid was used as a catalyst.

Bryant and Smith (13) developed a method in which the sample is added to a solution of hydroxylamine-hydrochloride in pyridine-bromphenol blue. After completion of the reaction the pyridine hydrochloride which is formed is titrated.

Thiobarbituric Acid Test - This test is concerned with the estimation of the oxidation of lipids through its formation of red color in a suitable solvent. The pigment is obtained as a result of a reaction between 2-TBA and malonic dialdehyde besides other aliphatic aldehydes.

Patton and Kurtz (108) have studied the reaction involved in the test and applied the test to detection of oxidation in milk fat. They found that malonic dialdehyde gave a strong test and that the TBA test is much more sensitive than the Kreis test. The latter did not begin to yield measurable colors until after very oxidized flavour appeared, whereas the TBA test gave good responses during the development

of perceptible rancidity. Methyl oleate hydroperoxides were also found to give the color reaction. Bernheim et al. (5) found that the color formed in the test is the result of a reaction between 2-TBA and oxidized unsaturated fatty acids or their degradation products. Briggs and Bryant (7) investigated various experimental factors of the TBA reaction with certain oxidized fatty acids and fatty esters. The procedure consists of heating the sample and the reagent together and then measuring the resulting red color in a spectrophotometer.

There are many variations of the TBA test; most of them being concerned with the adjustment to the particular fats or lipids under investigation. A method for fishery products was suggested by Yu and Sinnhuber (159) and later modified by the same authors (132). Others such as modifications for cereal and baked products (14), milk (33), butterfat (108), unsaturated fatty acids (157) frozen pork (130, 153), soybean oil, cottonseed oil and lard (129) were also developed.

Photochemical Methods - Oxidation-reduction indicators such as methylene blue have been utilized in different ways to estimate fat stability.

Greenbank and Holmes (54) used methylene blue. They found that the initial change in fats and oils could be detected through their action on the indicators in the presence of light. For this photochemical method the dye is mixed with the fat and the rate of reduction of the color of the dye indicates the rate of oxidation of the fat. Royce (125), however, found that the end point of the test was not satisfactory due to the formation of secondary color changes.

Coe (19) introduced a chemical procedure which he called chlorophyll value. It is based on the extent to which the natural fluorescence of chlorophyll was quenched when oxidized fat was added to it. The natural fluorescence of oils decreased as oxidation proceeded. French and Lundberg (41), however, did not find any stoichiometric relations between the quenching of the fluorescence and oxidation.

In 1943 Gudheim (59) constructed an accelerated light testing machine having approximately the same spectral distribution properties as sunlight to make a study on the effect of light on the odor and flavour of fat.

EXPERIMENTAL METHODS

The butter and butterfat were exposed to fluorescent light of the cool white variety which is the most commonly used type.

The state of oxidation of butter and butterfat was determined through the application of the peroxide test of Wheeler (158), the carbonyl test of Lappin and Clarke, as modified by Berry and McKerrigan (6), and determination of organoleptic properties by a small taste panel. The bleaching of the butter exposed to the light was recorded by photographs taken during the progress of bleaching.

Three sets of butter samples were tested. The first set was taken from different grocery stores at random. These samples were divided into two groups. The second group consisted of samples about which information as to distance from the light source and length of time of exposure were known. No such information was available about the first group.

The second set of samples was obtained unexposed from a local dairy. These samples were subjected to exposure to fluorescent lamps in the laboratory for various periods of time and at various distances from the light. They were exposed with parchment cover on and were tested periodically.

Before analysis, each sample of butter was divided into two parts. The first part consisted of layers of 1/4 inch thickness taken from the surface of the butter and designated (I) and the other

part consisted of the remainder after removal of the surfaces, and it is designated (II). This procedure was followed to determine the extent of penetration of the oxidation reaction to the interior of the samples.

The third set of samples was exposed to the lamp without wrappers. This set was divided into two groups. The first group, which is designated as (III), consisted of 1/4 inch of layers of butter and exposed to the light uncovered. The second group designated as (IV) was subjected to purification and dehydration and was poured into the petri dishes at approximately the same thickness as (III) and exposed to the light.

Chemical tests were done in triplicate and the average is presented in the tables.

The temperature used for exposure in the laboratory was 5°C. This is the usual keeping temperature used in grocery stores.

<u>Preparation of butterfat</u> (29) - Butterfat was prepared from butter by removal of the non-lipid material. The butter was melted, and washed repeatedly with warm water until the wash water became clear. The washed fat was then dried at the vacuum pump at about 50°C and subsequently filtered on a Buchner funnel.

<u>Organoleptic Evaluation</u> - All samples were tested by a small panel of tasters before chemical tests were run. A ranking method (36) was used and the following symbols were used to indicate the state of oxidation as judged by the panel:

- not oxidized

+ oxidized

++ highly oxidized

Bleaching of the butter was recorded photographically. The pictures were taken with the use of a green filter to obtain better contrast.

Peroxide Value (156) - Five grams of the fat were dissolved in 30 ml of acetic acid - chloroform (3:1). Saturated potassium iodide (1/2 ml) was added and the mixture was flushed with nitrogen for one minute. Thirty ml of distilled water and a few drops of starch indicator were added and the mixture titrated with 0.01 N sodium thiosulfate to a colorless end point.

Carbonyl Values - The Lappin and Clarke method as modified by Berry and McKerrigan (6) was used. This involved the formation of 2, 4 dinitrophenylhydrazones in benzene solution with trichloracetic acid as a catalyst. The absorbances at 430 and 460 mμ were used to determine total saturated and unsaturated carbonyl compounds.

150 mg of the fat were weighed in 25 ml volumetric flask and filled up to mark with carbonyl free benzene. Five ml of the solution was pipetted into a 50 ml volumetric flask and 3 ml of trichloracetic acid (6.5 g of trichloracetic dissolved in 150 ml of carbonyl-free benzene) and 5 ml of saturated solution of 2, 4 dinitrophenylhydrazine in benzene were added. The sample was incubated for 30 minutes at 60°C in a water bath. After cooling to room temperature, 10 ml of 4% potassium hydroxide in methyl alcohol were added and diluted to 50 ml with methyl alcohol. After 10 minutes, measurements of the carbonyls are taken at 430 and 460 mμ. A blank was determined.

RESULTS

Butter samples exposed to fluorescent light (cool white) showed marked deterioration as indicated by the results of chemical and organoleptic tests.

Table 1 outlines the results of the tests done on butter samples obtained at random from grocery stores. Organoleptic tests indicated that 10 out of the 12 samples of series (I) were highly oxidized, 1 slightly oxidized, and 1 not oxidized. The poor flavour exhibited by a majority of the samples seems to indicate that the butter samples had been exposed for some length of time before they were removed from the display cases. It is known that butter may oxidize during prolonged storage (15, 70, 122, 146), and it could be suggested that the butter may have been oxidized before it was put in the display cases. However, this possibility can be discounted as butter is carefully graded by official inspectors.

The results of the peroxide tests seem to indicate that there is little relationship between flavour and peroxide value. This agrees with the findings of Keeney and Doan (75) who found in their study of fat oxidation that there is little relationship between flavour and peroxide value. This is because peroxides are in themselves flavourless substances although they are the precursors of off-flavour compounds.

The samples of series (I) and (II) with the maximum total carbonyl values tasted only slightly oxidized while the samples of

series (I) and (II) with relatively low total carbonyl values tasted highly oxidized. Reports in the literature on flavour resulting from the autoxidation of milk and milk products agree that the main components of these flavours are carbonyl compounds (3, 40, 75, 76). However, the component or components directly responsible have not been identified with certainty. It appears that in this investigation, the presence of high carbonyl levels do not necessarily indicate that the compounds responsible for oxidized taste are present. The results confirm the findings of Day and Lillard (3) who concluded from their investigation of autoxidation of milk lipids that a complete spectrum of saturated and unsaturated carbonyl compounds was necessary to produce the flavours resulting from oxidation. The different flavours were believed to arise from the various ratios and levels of these compounds in the fats at certain times and under certain conditions.

Table 2 outlines the results of the measurement of the state of oxidation of butter samples exposed on supermarket display shelves. The samples were exposed for different lengths of time before testing. The flavour score indicated that the majority of the samples of series (I) were highly oxidized irrespective of the length of time of exposure. This finding is in agreement with the results of a study made by El-Negoumy _et al_. (34). They found in their study of the volatile components of autoxidized milk fat using gas chromatography, that a single fraction could reproduce the oxidized flavour in dairy products during the early stages of oxidation. That these samples are in the

initial stage of oxidation seems to be indicated by the results of the peroxide tests. There was relatively little variation in peroxide values of the samples of series (I) irrespective of the length of time of exposure, indicating that the samples were in their induction period. This is also true of the samples of series (II) although lower peroxide values were obtained.

The results of the tests done to follow the progress of oxidation of butter exposed in the laboratory at a distance of 10 inches from the light source are presented in Table 3. Exposure of 4 to 26 hours resulted in slightly oxidized flavour and after 2 days the samples tasted highly oxidized. The samples of series (II) did not have oxidized flavour after exposure from 2 to 10 hours and after 12 hours to 2 days' exposure they tasted slightly oxidized. Highly oxidized flavour was detected after 3 days to 4 weeks' exposure. The result of the peroxide tests demonstrated that the samples of series (I) were at the end of the induction period after two weeks' exposure when the value jumped to 29.74. The end of the induction period is indicated when a sudden increase in peroxide value occurs. This condition was also observed in the samples of series (II) although the peroxide values were not quite as high. It appears that the oxidation of the samples started at the surface and then gradually spread to the interior of the samples. This seems to support the findings of Dahle and Josephson (27) who found in his study of surface oxidation of butter that deterioration

of the samples starts at the surface before the interior of butter samples was affected. The results of the carbonyl tests seem to agree with the results of our previous experiments as shown in Tables 1 and 2. High levels of carbonyl compounds were not always accompanied by a highly oxidized flavour. The difficulty in detecting the carbonyl compounds responsible for oxidized flavour by chemical tests show that these compounds are probably highly specific. This was shown by Day and Lillard (28) who reported that oxidized flavour in milk fat is the result of combination of specific carbonyl compounds, namely, n-alkanals and alk-2-enals. They made this conclusion after finding that the qualitative composition of the mixture was the same regardless of the stage of oxidation of the fat.

Table 4 gives the results of the state of oxidation of butter samples exposed to fluorescent light at a distance of 20 inches. Samples of series (I) tasted highly oxidized after exposure for 18 hours. Samples of series (II) did not taste oxidized until after 10 hours exposure. Highly oxidized flavour was detected after exposure for 3 days. The peroxide values indicated that the induction period of the samples of series (I) ended after 2 weeks' exposure. The samples of series (II) showed that peroxides were still accumulating up to four weeks' exposure. Since the progress of a photochemical reaction depends on the amount

of energy absorbed by the substrate molecules, this observation appears to be in keeping with the distance between sample and light source. The results of the carbonyl tests closely parallels those of the flavour scores.

Table 5 gives the results of the tests done with butter samples exposed to fluorescent light at a distance of 30 inches. Organoleptic tests indicated that the samples of series (I) were slightly oxidized after 2 and up to 18 hours' exposure. They became highly oxidized thereafter. The results of peroxide tests indicated that the samples of series (I) were still in their induction period even after 4 weeks' exposure. Here again the role of the distance from the light is evident from the amount of the peroxides formed. The samples of series (II), giving lower peroxide values also indicated that they are in their induction period even after 4 weeks' exposure. The total carbonyl values showed the same trend as the development of oxidized flavour.

Table 6 gives the results of the tests done on butter and butterfat samples exposed at a distance of 10 inches from the light. The butterfat was prepared by melting of butter and repeated washing with warm water until the fat was clear and free from protein and phospholipids. It appears that more rapid oxidation occurred in the butterfat than in the butter because in two weeks the maximum amount of peroxides was formed in the butterfat whereas the

butter samples were still accumulating peroxides. According to Patton (106), triglycerides are relatively more stable when phospholipids are present in the aqueous phase of milk, and the phospholipids are preferentially oxidized. When water is absent the triglycerides are relatively more susceptible to oxidation, whereas the phospholipids are more stable. Since the butterfat was free of phospholipids the shorter induction period observed in the samples could be explained by this fact. The result of the carbonyl tests appears to indicate that high amount of carbonyls did not necessarily show off-flavour. This pattern of behaviour was also observed on the first set of butter samples. It is interesting to note that samples of almost the same amount of carbonyls gave off-flavour tastes of varying degrees. The specificity of the compounds responsible for oxidized flavour was the subject of several investigations (1, 3, 6, 10, 15, 36, 39, 40). Butter developed an oxidized flavour before the butterfat. This seems to agree with the findings of Tarassuk <u>et al</u>. (144) who observed that the origin of trainy flavour is in the phosphatide portion of the fat globule membrane material and that the flavour was produced by oxidation of the unsaturated fatty acids

of the phosphatides. The latter observation seems to hold true also for carbonyl formation in the samples. It was observed that higher amounts of carbonyls were present in the butter than in the butterfat. Since no phospholipids were present in the butterfat, butter could be expected to accumulate more carbonyls.

Table 8 gives the results of the tests done with butter and butterfat at a distance of 30 inches from the light. The induction period of the butter ended after exposure of 4 weeks. However, there was no appreciable change in the peroxide values of the samples of butterfat even after 4 weeks' exposure. This does not mean, however, that the samples did not reach the end of the induction period. More carbonyls seem to accumulate in the butter than in the butterfat. This is a pattern observed also in our previous experiment as shown by the results presented in Table 7. It is observed, however, that the butter developed oxidized flavour before the butterfat.

It has been reported that the development of rancidity in butter is accompanied by bleaching of carotene (69). The bleaching

of the butter in our experiment is presented photographically.

Figure 1 shows butter as it is exposed to light in the display shelves of a typical supermarket. Figure 2 shows the butter samples wrapped as they were exposed to light in the laboratory. Figures 3 to 8 show the butter samples after they were exposed to light for one week, the bleaching of the butter is indicated by the colour contrast between the control and the exposed sample.

Figures 9 to 14 show the butter samples after they were exposed for 2 weeks. The bleaching of the butter is more intense. The part of the butter covered by the dark-coloured trademark on the parchment seemed to have been protected somewhat. The protection of the parchment where it overlaps can also be seen.

Figures 15 to 20 show the butter samples after they were exposed to light for 3 weeks. Colour contrast between the exposed and the protected parts is more pronounced and bleaching is more distinct. The distance from the light seems to play a role since the contrast in the intensity of bleaching can be observed at different distances. There is more distinct and intense bleaching of the butter exposed at shorter distance for the same amount of time.

Figures 21 to 26 show the intensity of bleaching after four weeks' exposure. In this case even the darker part of the parchment affords little protection as indicated by Figure 21, which represents the shortest distance. At a greater distance, however, the protection of the dark-coloured part of the parchment paper is still evident, indicating that distance plays a role in butter bleaching.

TABLE 1. Measurement of state of oxidation of butter samples obtained from supermarket display shelves.

Sample No.	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmoles/kg) Saturated		Carbonyls (Mmoles/kg) Unsaturated		Total Carbonyls	
	I	II	I	II	I	II	I	II	I	II
1	++	++	4.00	2.00	5.78	1.80	5.28	6.84	11.06	8.64
2	++	+	1.30	0.76	4.25	5.19	2.97	2.96	7.32	8.15
3	++	-	1.20	0.92	2.37	3.30	1.37	2.23	3.74	5.53
4	-	-	0.56	0.39	3.06	2.25	3.05	0.85	6.11	3.10
5	+	+	1.19	0.64	14.80	17.94	5.80	6.11	20.60	24.05
6	++	+	0.79	0.46	1.62	1.90	3.94	3.72	5.56	5.62
7	++	+	1.49	0.83	5.18	4.46	3.82	2.58	9.00	7.04
8	++	++	3.64	1.42	3.59	3.72	2.46	2.48	6.05	6.20
9	++	++	2.51	1.62	3.59	1.60	2.94	3.98	6.53	5.58
10	++	+	2.15	3.68	3.14	6.51	2.61	1.55	5.75	8.06
11	++	+	2.00	0.84	1.30	1.53	2.82	5.04	4.12	6.57
12	++	++	4.64	0.80	2.69	1.11	2.41	1.02	5.10	2.13

(I) surface layers 1/4 inch thick
(II) the butter remaining after removal of the surface layers
(- denotes not oxidized, + slightly oxidized and ++ highly oxidized)

TABLE 2. Measurement of state of oxidation of butter samples exposed on supermarket display shelves.

Sample No.	Days Exposed	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmole/kg) Saturated		Carbonyls (Mmole/kg) Unsaturated		Total Carbonyls	
		I	II	I	II	I	II	I	II	I	II
1	1	++	++	1.53	0.47	0.13	0.09	0.12	0.08	0.25	0.17
2	1	++	+	1.33	1.66	0.11	0.07	0.10	0.09	0.21	0.16
3	1	++	-	2.41	1.46	0.09	0	0.04	0	0.13	0
4	1	+	-	2.92	1.12	0.86	0.03	0.29	0.06	1.15	0.90
5	1	+	-	1.95	0.61	0.63	0	0.22	0	0.85	0
6	1	++	+	1.81	0.37	0.98	0.31	0.44	0.15	1.42	0.46
7	1	++	+	1.88	0.78	0.59	0.53	0.49	0.74	1.08	1.27
8	2	+	-	0.95	0.44	2.37	2.03	1.08	0.91	3.45	2.94
9	2	+	+	1.87	0.38	0.18	0.30	0.20	0.32	0.38	0.62
10	2	++	+	1.57	0.47	0.80	0.70	0.07	0.74	0.87	1.44
11	2	++	+	2.67	0.98	0.48	0.82	0.99	0.51	1.47	1.33
12	2	+	+	2.66	1.18	0.23	0.16	0.17	0.15	0.40	0.31
13	2	++	-	2.11	0.78	1.32	0.16	1.34	0.58	1.66	0.74
14	3	++	+	1.23	0.32	0.19	0.44	0.48	0.22	0.67	0.66
15	3	++	+	2.14	0.39	0.28	0.24	0.24	0.45	0.52	0.69
16	3	+	+	2.66	1.07	0.30	0.30	0.29	0.25	0.59	0.55

(I) surface layers 1/4 inch thick
(II) the butter remaining after removal of the surface layer
(- denotes oxidized, + slightly oxidized and ++ highly oxidized)

Before exposure the butter samples were first grade with no oxidized flavour.

TABLE 3. Progress of oxidation of butter exposed to fluorescent light at a distance of 10 inches.

Sample No.	Time Exposed	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmole/kg) Saturated		Carbonyls (Mmole/kg) Unsaturated		Total Carbonyls	
		I	II	I	II	I	II	I	II	I	II
1	2 hrs	-	-	0.75	0.53	2.41	0.78	2.40	1.01	4.81	1.79
2	4 hrs	+	-	1.70	0.69	0.73	0.45	1.15	0.60	1.88	1.05
3	6 hrs	+	-	0.95	0.75	6.74	4.25	7.64	4.93	14.38	9.18
4	8 hrs	+	-	1.45	0.65	1.87	1.84	1.62	2.99	3.49	4.83
5	10 hrs	+	-	1.17	0.55	1.34	3.70	1.81	1.77	3.15	5.47
6	12 hrs	+	+	0.87	0.46	0.68	2.69	2.38	6.98	3.06	9.67
7	16 hrs	+	+	0.55	1.30	2.56	4.88	5.88	3.91	8.44	8.79
8	18 hrs	+	+	0.57	0.60	17.28	6.25	4.30	3.03	21.58	9.28
9	24 hrs	+	+	1.93	0.53	16.34	8.64	2.88	3.21	19.22	11.85
10	26 hrs	+	+	1.90	0.76	1.90	0.76	4.39	3.23	6.29	3.99
11	2 days	++	+	2.67	1.29	0.81	0.17	0.33	0.53	1.14	0.70
12	3 days	++	++	3.76	1.74	0.76	0.78	0.95	0.95	1.71	1.73
13	4 days	++	++	2.26	1.32	0.24	0.06	0.63	0.40	0.87	0.46
14	5 days	++	++	2.23	0.83	0.70	15.85	9.98	1.66	10.68	17.51
15	1 week	++	++	1.76	0.57	3.42	5.35	5.32	8.38	8.74	13.73
16	2 weeks	++	++	5.66	1.05	1.00	0.54	4.50	12.19	5.50	12.73
17	3 weeks	++	++	29.74	4.80	0.53	0.69	0.97	0.23	1.50	0.92
18	4 weeks	++	++	12.75	3.25	7.07	9.42	13.18	7.78	20.25	17.20

(I) surface layers 1/4 inch thick
(II) the butter remaining after removal of the surface layer
(- denotes not oxidized, + slightly oxidized and ++ highly oxidized)

Before exposure, the butter samples were first grade with no oxidized flavour.

TABLE 4. Progress of oxidation of butter exposed to fluorescent light at a distance of 20 inches.

Sample No.	Time Exposed	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmoles/kg) Saturated		Carbonyls (Mmoles/kg) Unsaturated		Total Carbonyls	
		I	II	I	II	I	II	I	II	I	II
1	2 hrs	+	-	0.82	0.42	0.81	0.63	0.81	0.88	1.62	1.51
2	4 hrs	+	-	0.89	0.86	0.70	0.55	0.98	1.66	1.68	2.21
3	6 hrs	+	-	0.77	0.57	0.71	0.31	0.92	0.42	1.63	0.73
4	8 hrs	+	-	0.76	0.34	0.27	0.43	0.49	0.15	0.76	0.58
5	10 hrs	+	+	0.49	1.32	0.76	0.37	0.16	0.10	0.92	0.47
6	12 hrs	+	+	1.00	0.53	0.72	0.03	4.01	5.19	4.73	5.22
7	16 hrs	+	+	0.49	0.45	0.26	0.27	0.08	0.39	0.34	0.66
8	18 hrs	++	+	0.77	0.66	0.85	0.90	0.39	0.70	1.24	1.60
9	24 hrs	++	+	0.35	0.22	0.11	0.97	0.26	0.15	0.37	1.12
10	26 hrs	++	+	2.18	0.66	2.35	2.28	0.82	0.94	3.17	3.22
11	2 days	++	+	1.85	0.69	2.31	1.62	0.85	0.79	3.16	2.41
12	3 days	++	++	2.65	1.29	0.05	0.16	3.08	3.69	3.13	3.85
13	4 days	++	++	2.72	1.02	0.72	0.03	4.01	5.19	4.73	5.22
14	5 days	++	++	7.12	3.52	1.05	2.82	4.82	2.82	5.87	5.64
15	1 week	++	++	3.19	2.78	3.42	5.35	5.35	8.38	8.77	13.73
16	2 weeks	++	++	19.58	2.24	0.22	0.10	1.35	0.57	1.57	0.67
17	3 weeks	++	++	7.76	3.34	3.20	1.97	7.32	6.16	10.52	8.13
18	4 weeks	++	++	9.85	2.12	0.22	2.12	0.40	0.23	0.62	2.35

(I) surface layers 1/4 inch thick
(II) the butter remaining after removal of the surface layer
(- denotes not oxidized, + slightly oxidized and ++ highly oxidized)

Before exposure, the butter samples were first grade with no oxidized flavour.

TABLE 5. Progress of oxidation of butter exposed to fluorescent light at a distance of 30 inches.

Sample No.	Time Exposed	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmoles/kg) Saturated		Carbonyls (Mmoles/kg) Unsaturated		Total Carbonyls	
		I	II	I	II	I	II	I	II	I	II
1	2 hrs	+	-	0.36	0.19	2.19	3.28	1.78	3.61	3.79	6.89
2	4 hrs	+	-	0.25	0.19	3.29	2.68	5.53	0.38	8.82	3.06
3	6 hrs	+	-	1.17	0.30	0.46	0.05	0.38	3.40	9.84	3.45
4	8 hrs	+	+	0.97	0.96	0.33	0.48	3.72	4.07	4.05	4.55
5	10 hrs	+	+	0.97	1.50	0.74	0.70	0.13	0.26	0.87	0.96
6	12 hrs	+	+	0.98	0.55	1.78	0.70	6.02	3.98	7.80	4.68
7	16 hrs	+	+	0.24	0.32	0.05	0.47	0.05	0.77	0.10	1.24
8	18 hrs	+	+	0.42	0.66	0.18	0.08	0.21	0.76	0.39	0.84
9	24 hrs	++	+	0.24	0.32	0.22	0.10	0.10	0.08	0.32	0.81
10	26 hrs	++	+	1.78	1.34	2.45	0.05	0.89	0.98	3.34	1.03
11	2 days	++	+	2.20	1.34	2.09	0.08	0.82	0.82	2.91	0.90
12	3 days	++	++	2.37	0.89	1.49	0.75	5.05	4.28	6.54	5.03
13	4 days	++	++	2.22	0.77	0.42	2.30	3.62	3.27	4.04	5.57
14	5 days	++	++	3.52	2.13	1.05	1.32	3.33	2.13	4.38	3.45
15	1 week	++	++	3.81	0.11	2.96	2.97	4.25	5.19	7.31	8.16
16	2 weeks	++	++	2.39	1.23	3.46	0.40	11.80	28.93	15.26	29.33
17	3 weeks	++	++	2.13	1.78	17.32	2.51	27.17	1.29	44.49	3.80
18	4 weeks	++	++	3.77	1.41	0.22	0.08	0.40	0.23	0.62	1.03

(I) surface layers 1/4 inch thick
(II) the butter remaining after removal of the surface layer
(- denotes not oxidized, + slightly oxidized and ++ highly oxidized)

Before exposure, the butter samples were first grade with no oxidized flavour.

TABLE 6. Progress of oxidation of uncovered butter and butterfat samples exposed to fluorescent light at a distance of 10 inches.

Sample No.	Time Exposed	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmoles/kg) Saturated		Carbonyls (Mmoles/kg) Unsaturated		Total Carbonyls	
		III	IV	III	IV	III	IV	III	IV	III	IV
1	2 hrs	+	-	0.69	0.62	0.93	0.85	5.09	5.79	6.02	6.64
2	4 hrs	+	+	0.93	0.93	2.05	3.34	2.37	1.59	4.42	4.93
3	6 hrs	+	+	1.26	1.28	0.30	0.11	6.71	8.51	7.01	8.62
4	8 hrs	+	+	1.01	0.71	0.27	0.24	4.73	5.09	5.00	5.33
5	10 hrs	++	+	0.56	0.53	0.05	2.31	4.19	1.76	4.24	4.07
6	12 hrs	++	++	1.52	0.95	2.02	0.05	2.26	4.18	4.28	4.13
7	16 hrs	++	++	1.13	1.32	0.74	3.64	4.22	1.43	4.96	5.07
8	18 hrs	++	++	2.19	1.00	0.64	1.06	7.66	4.25	8.30	5.31
9	24 hrs	++	++	3.36	1.93	1.86	1.44	2.81	5.52	4.67	6.96
10	26 hrs	++	++	2.04	2.09	0.43	1.53	9.28	5.65	9.71	7.18
11	2 days	++	++	4.65	2.74	1.01	0.82	6.80	4.67	7.81	5.49
12	3 days	++	++	3.20	2.32	0.59	0.67	7.05	6.13	7.64	6.80
13	4 days	++	++	7.55	6.71	1.13	1.66	5.20	5.28	6.33	6.94
14	5 days	++	++	4.09	3.48	2.11	1.98	5.84	6.19	7.95	8.17
15	1 week	++	++	8.44	6.63	2.80	1.66	7.14	5.00	9.94	6.66
16	2 weeks	++	++	12.47	12.45	2.11	1.98	5.84	6.19	7.95	8.17
17	3 weeks	++	++	13.39	9.87	6.76	6.87	18.78	30.65	25.54	37.52
18	4 weeks	++	++	8.15	6.75	9.17	13.41	13.23	13.41	22.40	26.82

(III) surface layers 1/4 inch thick
(IV) purified sample of butterfat and exposed uncovered in petri dish

Before exposure, the butter samples were first grade with no oxidized flavour.

TABLE 7. Progress of oxidation of uncovered butter and butterfat samples exposed to fluorescent light at a distance of 20 inches.

Sample No.	Time Exposed	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmoles/kg) Saturated		Carbonyls (Mmoles/kg) Unsaturated		Total Carbonyls	
		III	IV	III	IV	III	IV	III	IV	III	IV
1	2 hrs	+	-	0.46	0.48	0.92	1.20	7.24	7.78	8.16	8.98
2	4 hrs	+	-	0.46	0.45	1.40	0.58	5.38	4.82	6.78	5.40
3	6 hrs	+	-	0.57	0.57	1.26	0.30	7.71	6.76	8.97	7.06
4	8 hrs	+	+	0.91	0.73	0.30	0.70	6.67	4.01	6.97	4.71
5	10 hrs	+	+	0.44	0.45	0.58	1.13	2.69	2.41	3.27	3.54
6	12 hrs	+	+	0.46	0.47	1.08	0.99	2.36	3.98	3.44	4.97
7	16 hrs	++	+	2.05	2.13	2.75	2.70	3.91	4.17	6.66	6.87
8	18 hrs	++	++	0.97	1.03	0.68	0.73	8.05	7.89	8.93	8.62
9	24 hrs	++	++	1.51	1.01	0.62	0.30	7.22	3.78	7.84	4.08
10	26 hrs	++	++	1.11	1.52	0.29	0.76	6.52	9.74	6.81	10.50
11	2 days	++	++	2.17	2.62	18.79	0.03	28.64	6.41	47.43	6.44
12	3 days	++	++	1.68	1.58	0.30	4.42	6.84	31.96	7.14	36.38
13	4 days	++	++	4.71	3.34	1.07	0.11	9.01	4.40	10.08	4.51
14	5 days	++	++	1.67	1.87	0.46	0.24	5.58	4.59	6.02	4.83
15	1 week	++	++	4.14	3.55	0.04	0.03	2.60	5.70	2.64	5.73
16	2 weeks	++	++	8.43	8.43	1.50	0.84	9.70	6.20	10.20	7.04
17	3 weeks	++	++	10.17	12.06	13.61	5.00	0.78	10.32	14.39	15.32
18	4 weeks	++	++	11.74	11.11	4.11	3.19	11.41	11.61	15.52	14.80

(III) surface layers 1/4 inch thick
(IV) purified sample of butterfat and exposed uncovered in petri dish

Before exposure, the butter samples were first grade with no oxidized flavour.

TABLE 8. Progress of oxidation of uncovered butter and butterfat samples exposed to fluorescent light at a distance of 30 inches.

Sample No.	Time Exposed	Flavour Score		Peroxide Value Meq/kg		Carbonyls (Mmoles/kg) Saturated		Carbonyls (Mmoles/kg) Unsaturated		Total Carbonyls	
		III	IV	III	IV	III	IV	III	IV	III	IV
1	2 hrs	-	-	0.37	0.38	2.18	2.24	5.03	9.50	7.21	11.74
2	4 hrs	-	-	0.50	0.45	1.23	0.88	1.76	4.51	2.99	5.39
3	6 hrs	+	-	0.45	0.48	0.58	1.01	6.01	7.09	6.59	8.10
4	8 hrs	+	-	2.16	1.09	2.41	0.73	0.73	1.18	3.14	1.91
5	10 hrs	+	+	1.28	1.06	0.60	0.93	1.18	4.28	1.78	5.21
6	12 hrs	+	+	0.64	0.57	0.27	0.64	4.06	2.55	4.33	3.19
7	16 hrs	++	+	1.10	1.09	1.04	0.04	4.70	6.67	5.74	6.71
8	18 hrs	++	+	1.02	0.97	0.30	0.32	6.80	7.30	7.10	7.62
9	24 hrs	++	++	1.10	1.33	4.20	1.48	8.85	5.00	13.05	6.48
10	26 hrs	++	++	1.07	1.19	0.33	0.05	7.59	6.00	7.92	6.05
11	2 days	++	++	2.18	2.17	0.42	2.30	3.62	3.27	4.02	5.57
12	3 days	++	++	1.62	1.82	3.93	3.22	7.12	4.57	11.04	4.79
13	4 days	++	++	3.59	3.14	0.47	0.46	4.00	3.98	4.47	4.44
14	5 days	++	++	1.03	1.87	1.00	0.26	4.59	5.72	5.59	5.98
15	1 week	++	++	2.23	3.17	1.11	0.73	5.17	4.20	6.28	9.93
16	2 weeks	++	++	2.55	2.79	2.11	1.98	5.84	6.19	7.95	8.17
17	3 weeks	++	++	3.20	4.09	6.76	6.87	18.78	30.65	25.54	37.52
18	4 weeks	++	++	6.26	3.89	6.73	3.41	9.55	7.84	16.28	11.25

(III) surface layers 1/4 inch thick
(IV) purified sample of butterfat and exposed uncovered in petri dish

Before exposure, the butter samples were first grade with no oxidized flavour.

Fig. 1. Typical butter display shelf in a supermarket.

Fig. 2. Butter in parchment wrap for exposure test.
Note side (a) and side (b).

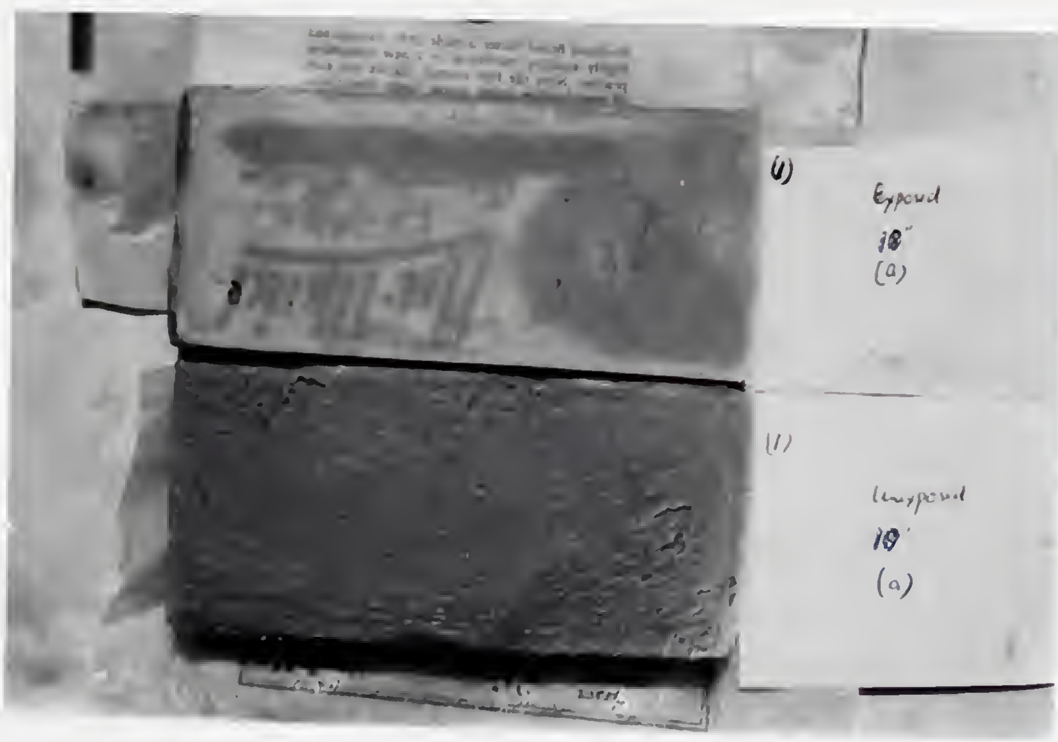

Fig. 3. Butter exposed for one week at a distance of 10 inches from the light, side (a).

Fig. 4. Butter exposed for one week at a distance of 10 inches from the light, side (b).

Fig. 5. Butter exposed for one week at a distance of 20 inches from the light, side (a).

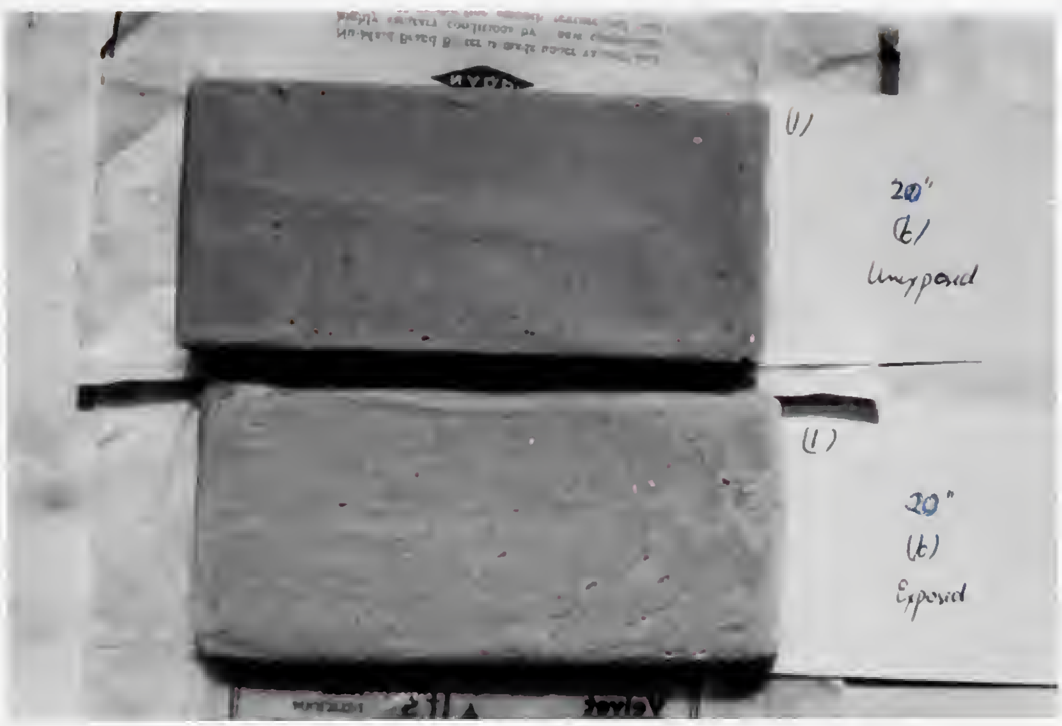

Fig. 6. Butter exposed for one week at a distance of 20 inches from the light, side (b).

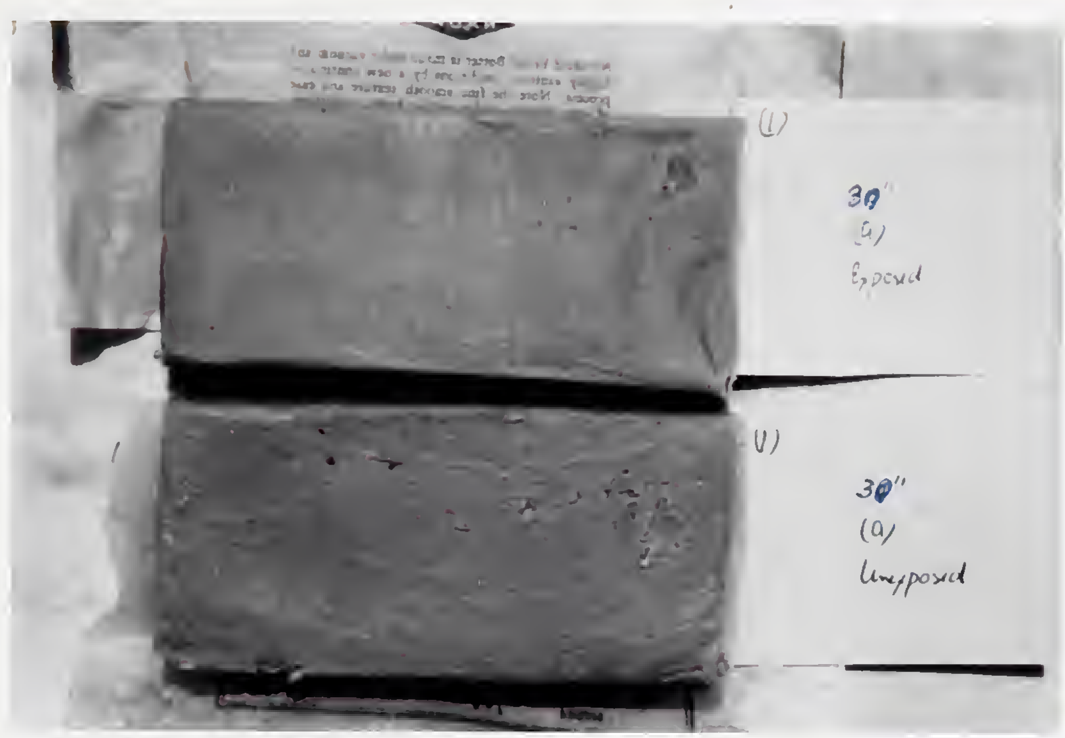

Fig. 7. Butter exposed for one week at a distance of 30 inches from the light, side (a).

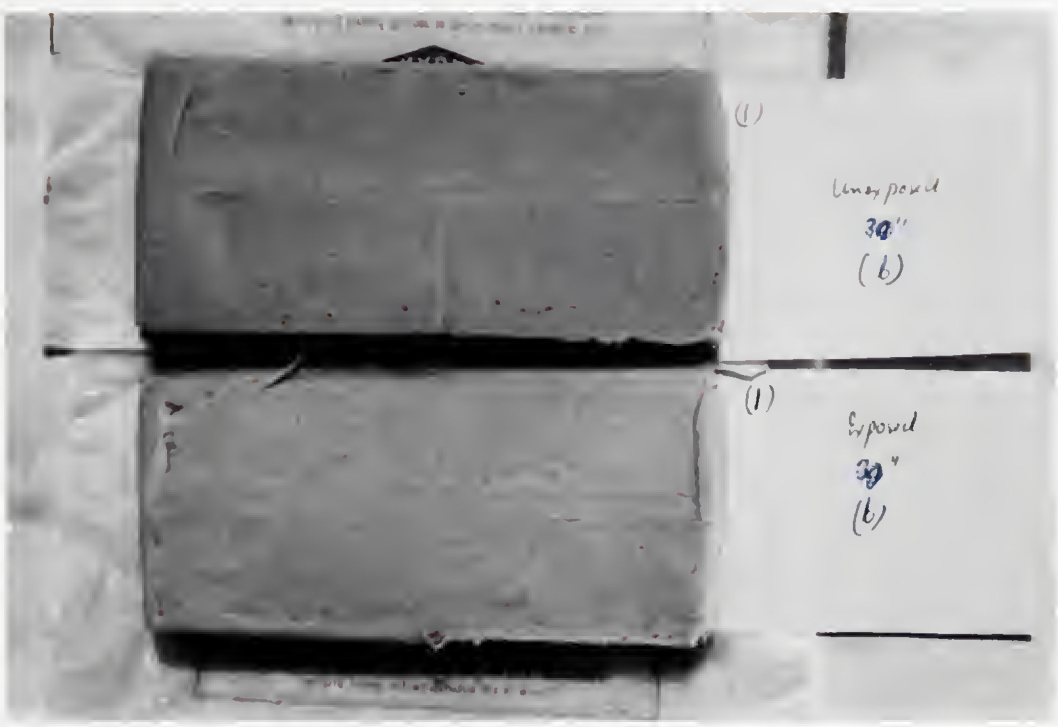

Fig. 8. Butter exposed for one week at a distance of 30 inches from the light, side (b).

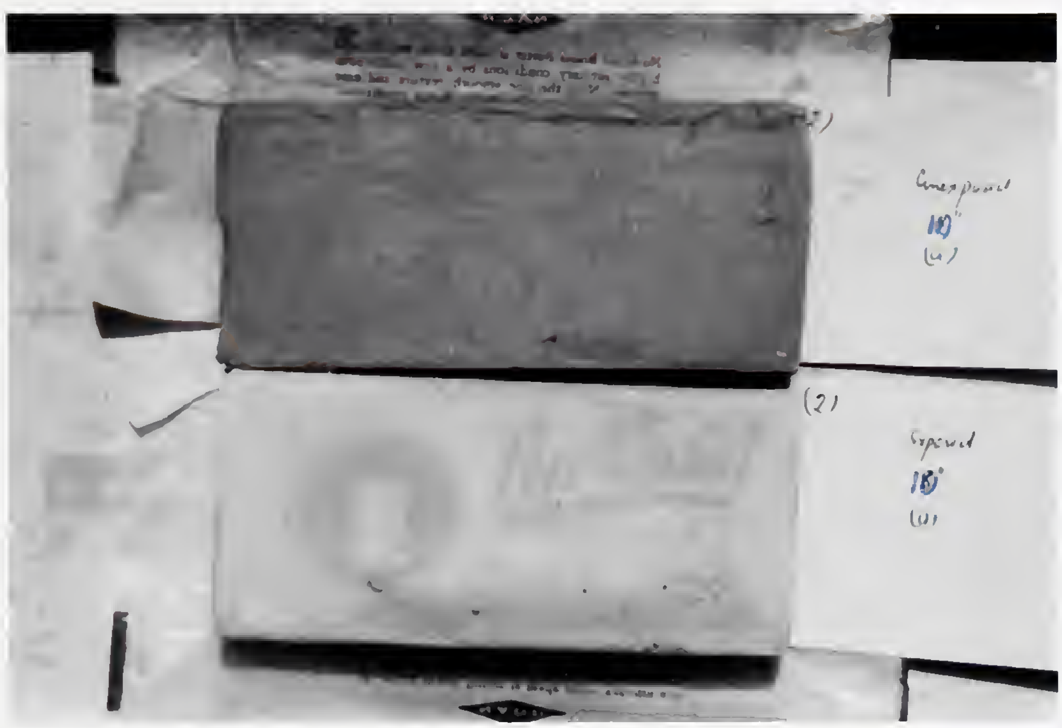

Fig. 9. Butter exposed for two weeks at a distance of 10 inches from the light, side (a).

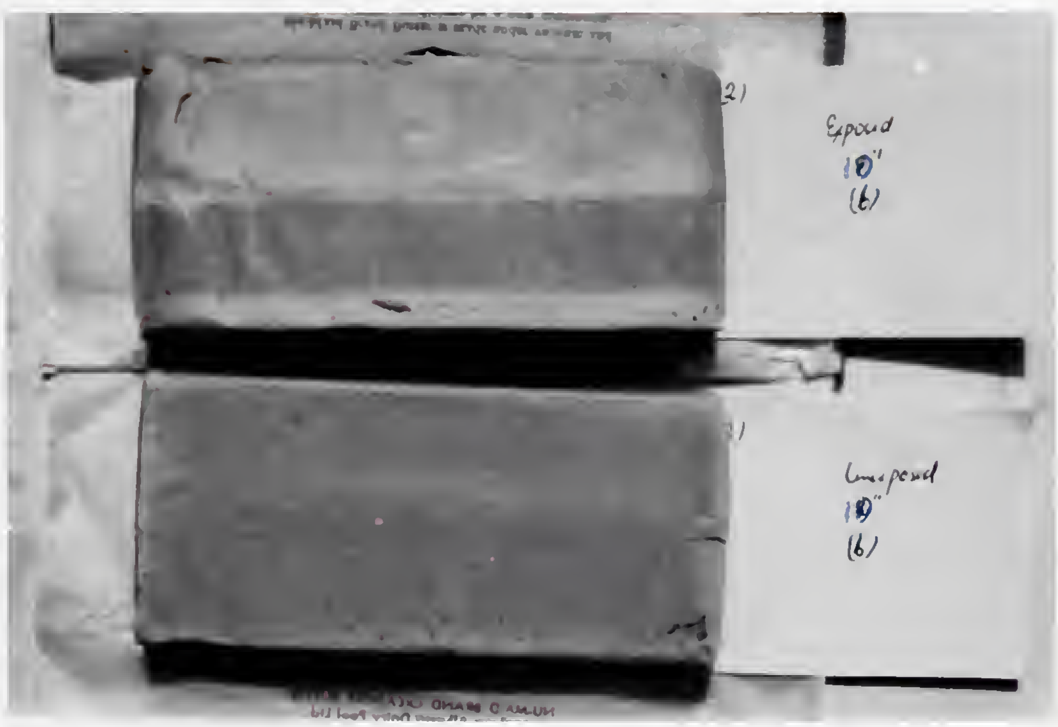

Fig. 10. Butter exposed for two weeks at a distance of 10 inches from the light, side (b).

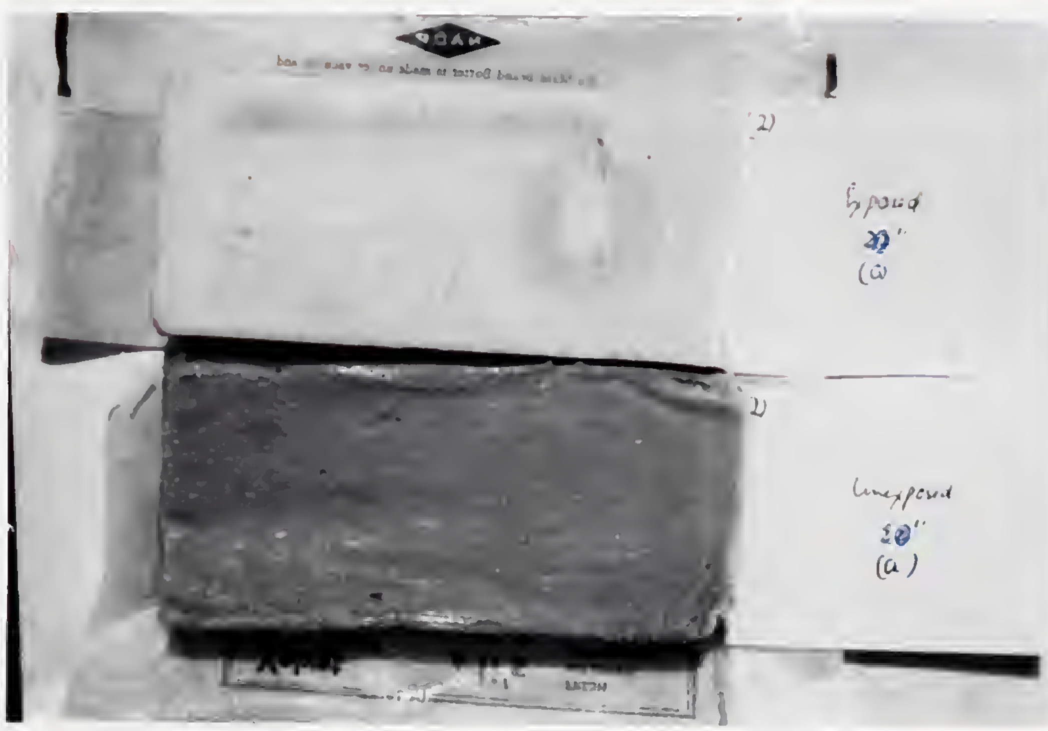

Fig. 11. Butter exposed for two weeks at a distance of 20 inches from the light, side (a).

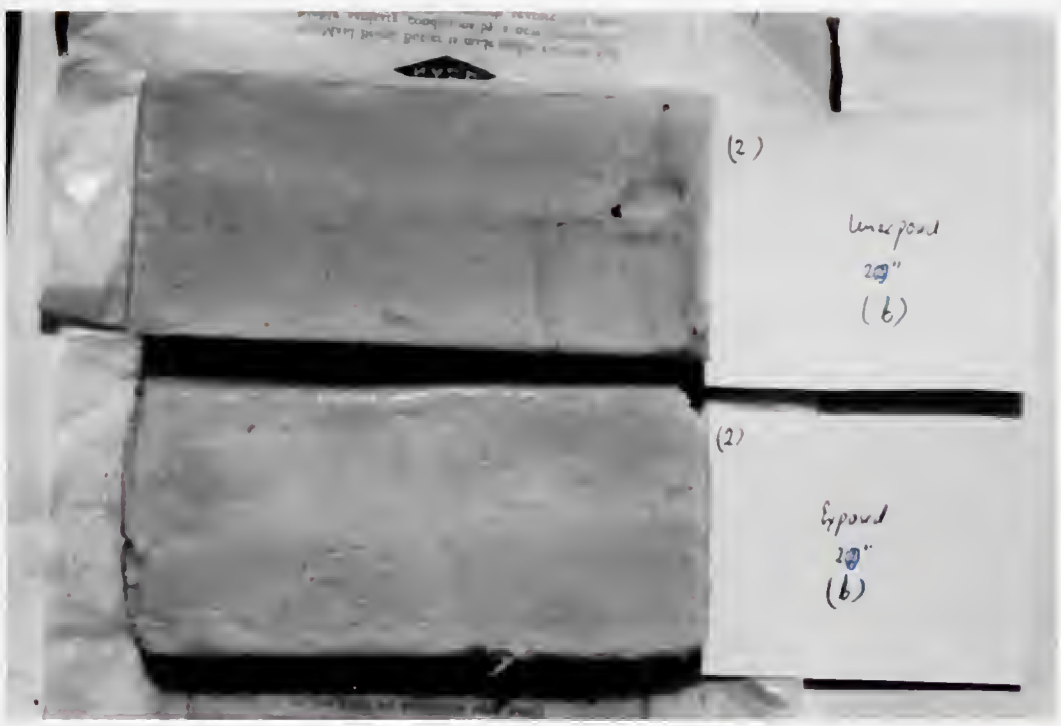

Fig. 12. Butter exposed for two weeks at a distance of 20 inches from the light, side (b).

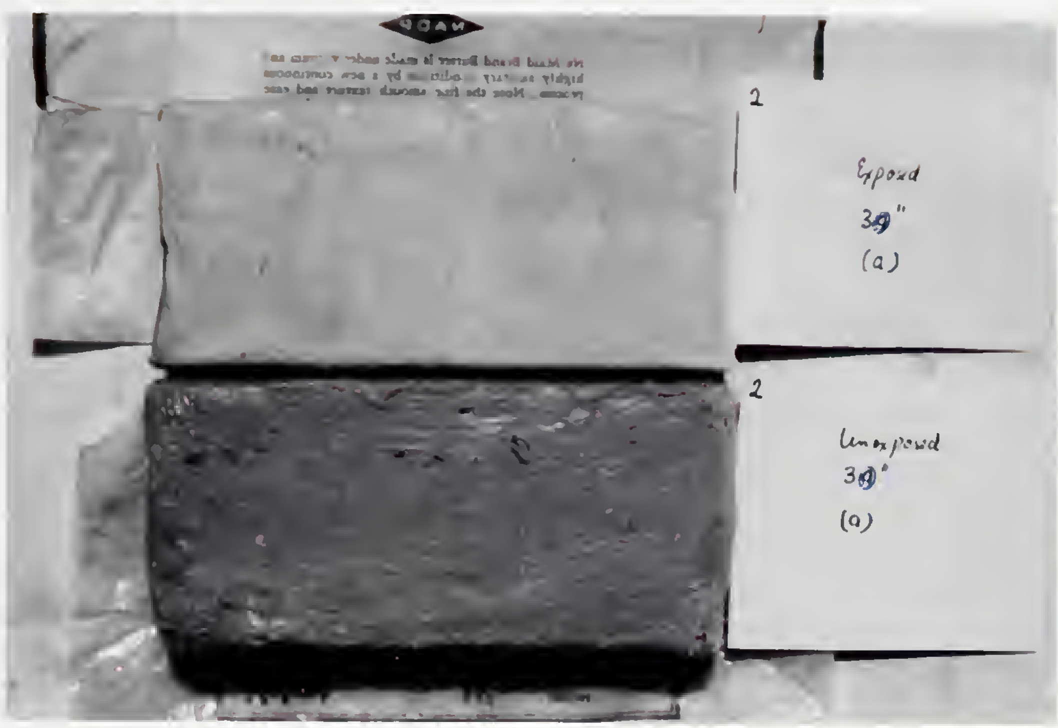

Fig. 13. Butter exposed for two weeks at a distance of 30 inches from the light, side (a).

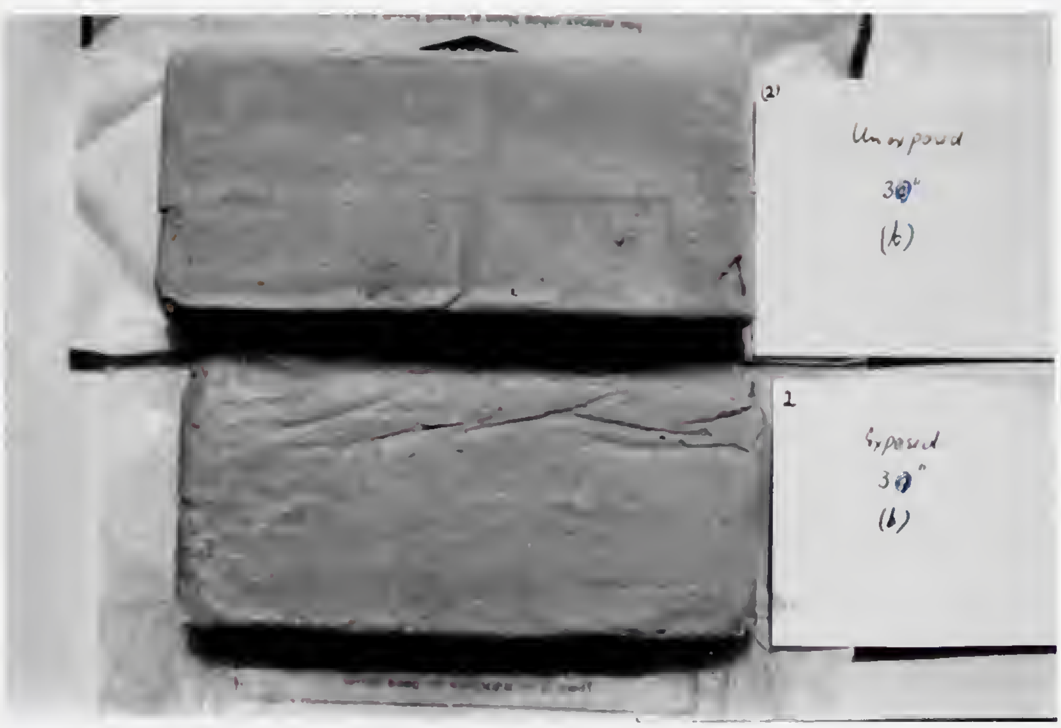

Fig. 14. Butter exposed for two weeks at a distance of 30 inches from the light, side (b).

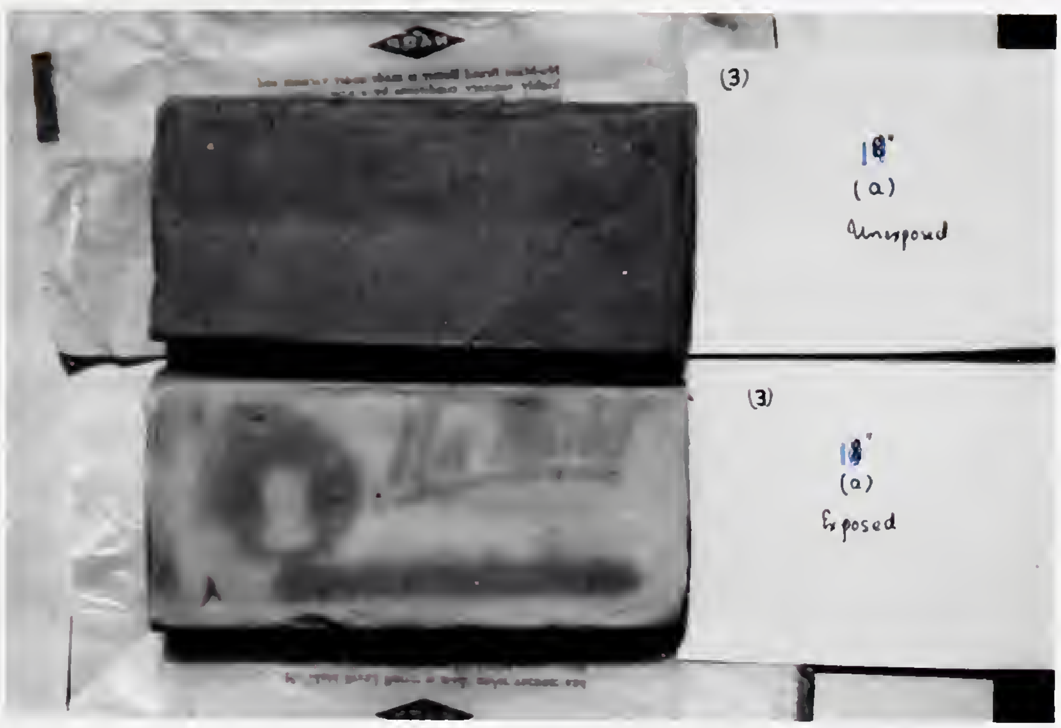

Fig. 15. Butter exposed for three weeks at a distance of 10 inches from the light, side (a).

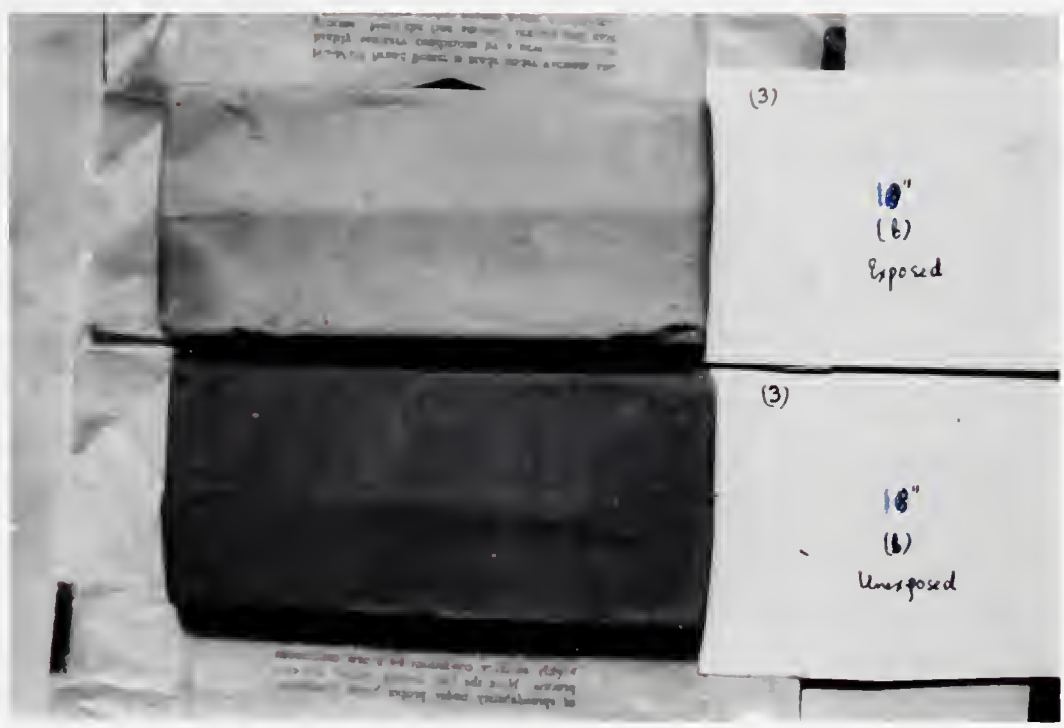

Fig. 16. Butter exposed for three weeks at a distance of 10 inches from the light, side (b).

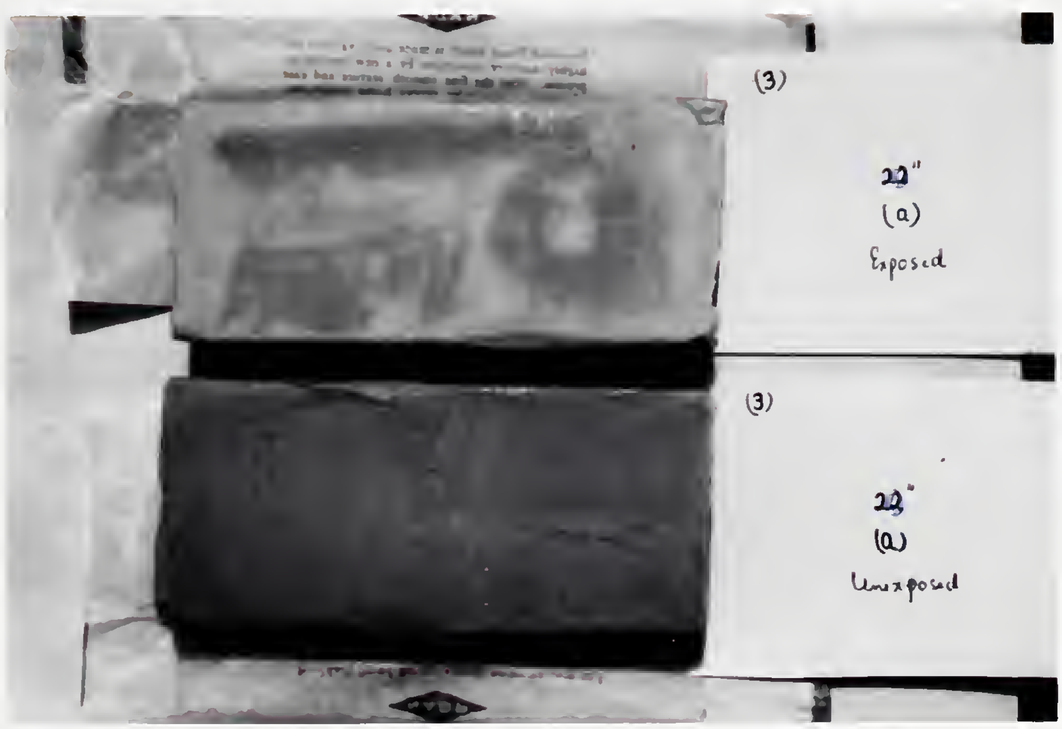

Fig. 17. Butter exposed for three weeks at a distance of 20 inches from the light, side (a).

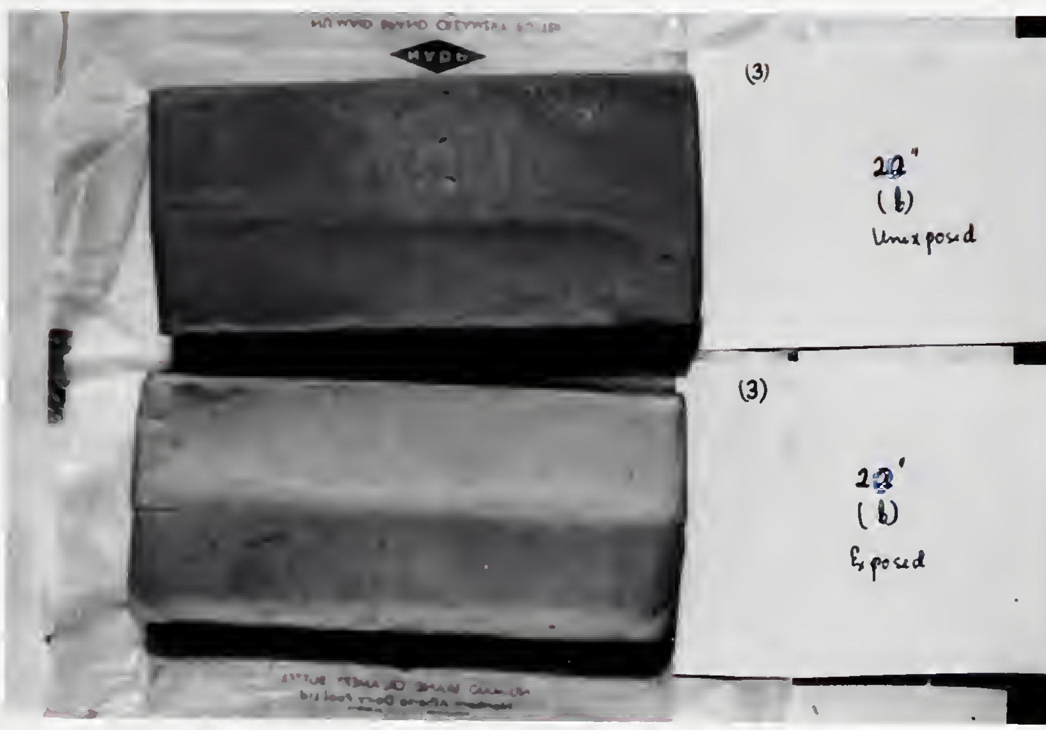

Fig. 18. Butter exposed for three weeks at a distance of 20 inches from the light, side (b).

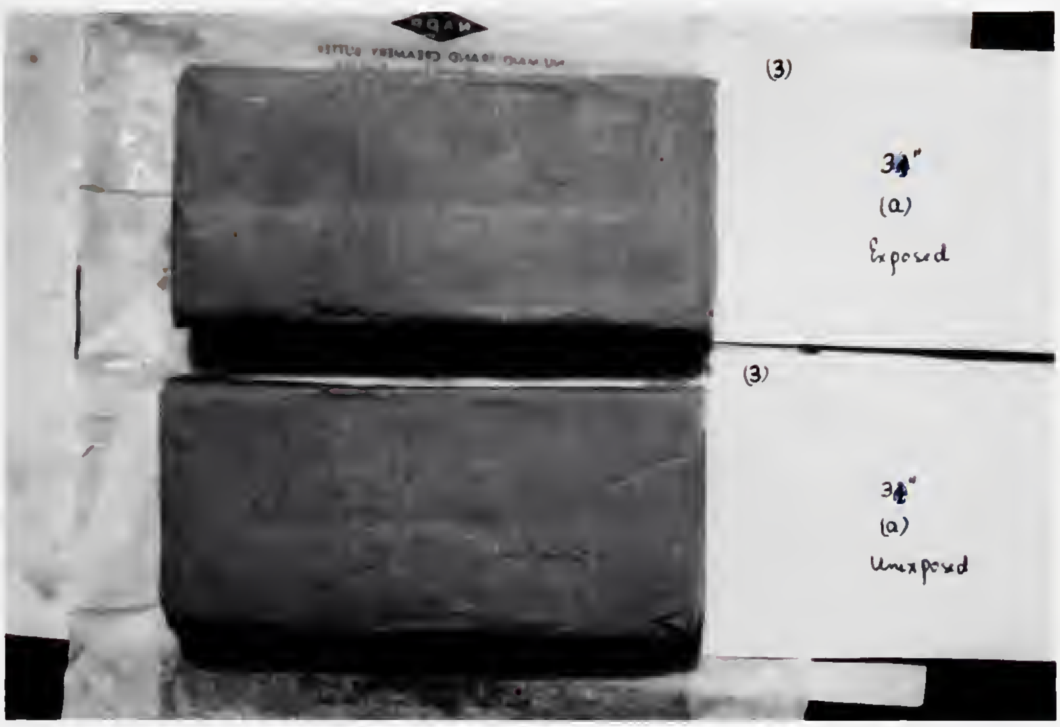

Fig. 19. Butter exposed for three weeks at a distance of 30 inches from the light, side (a).

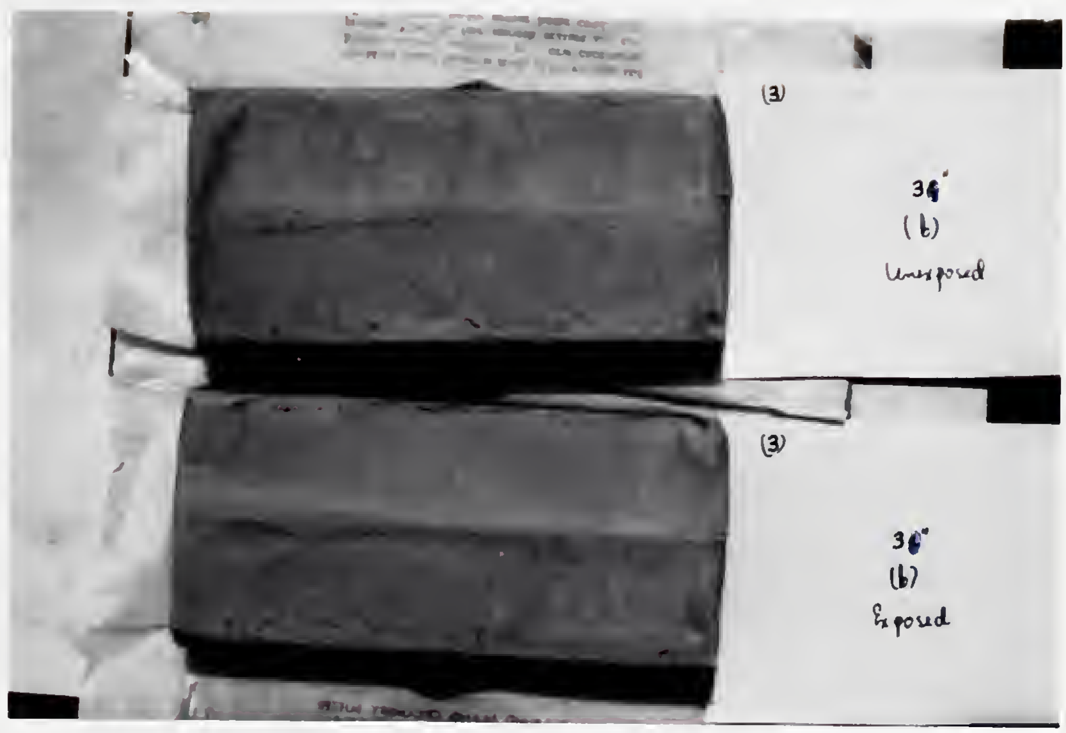

Fig. 20. Butter exposed for three weeks at a distance of 30 inches from the light, side (b).

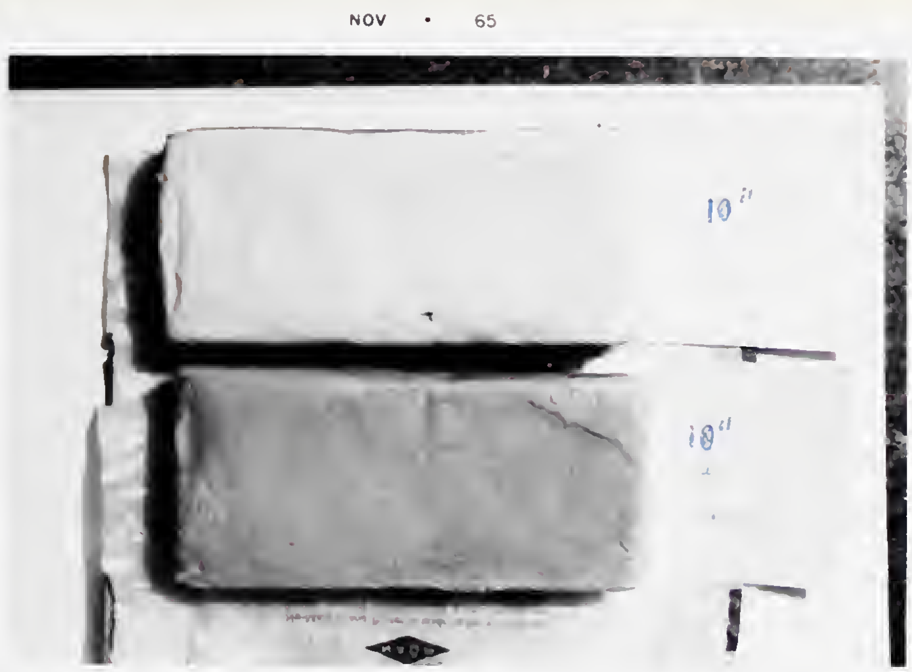

Fig. 21. Butter exposed for four weeks at a distance of 10 inches from the light, side (a).

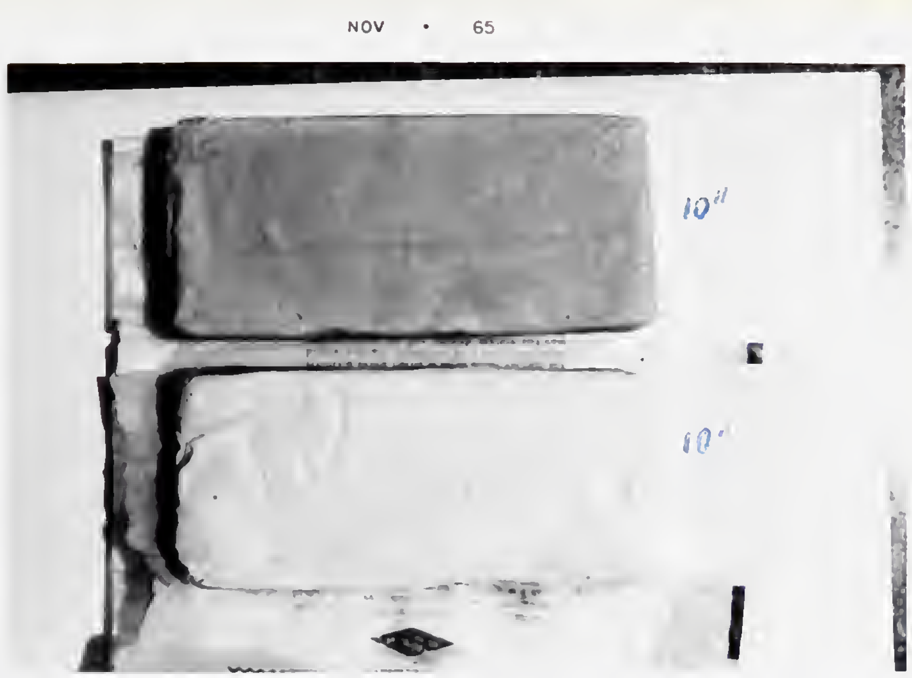

Fig. 22. Butter exposed for four weeks at a distance of 10 inches from the light, side (b).

Fig. 23. Butter exposed for four weeks at a distance of 20 inches from the light, side (a).

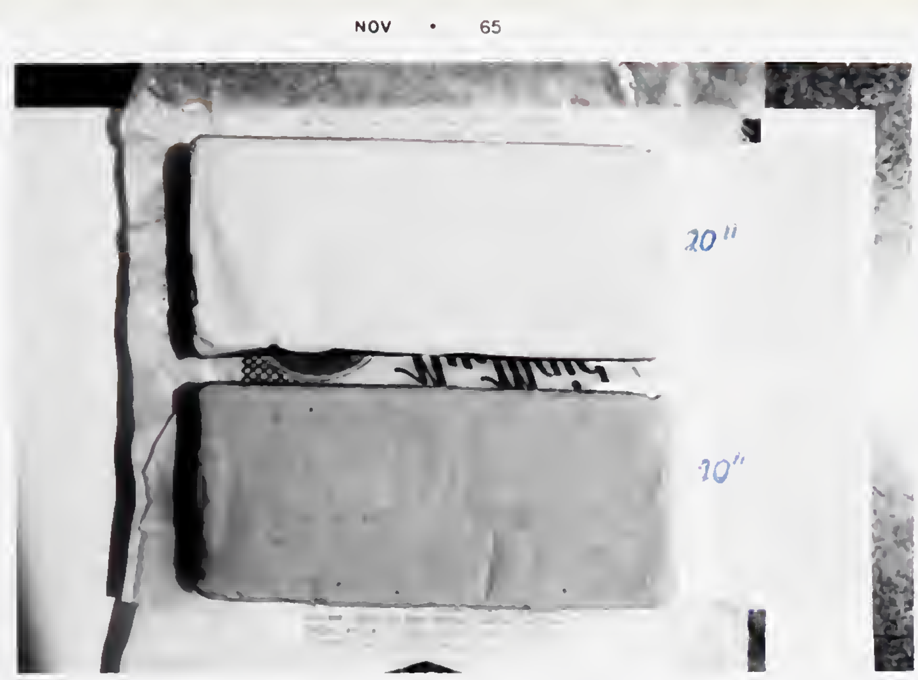

Fig. 24. Butter exposed for four weeks at a distance of 20 inches from the light, side (b).

Fig. 25. Butter exposed for four weeks at a distance of 30 inches from the light, side (a).

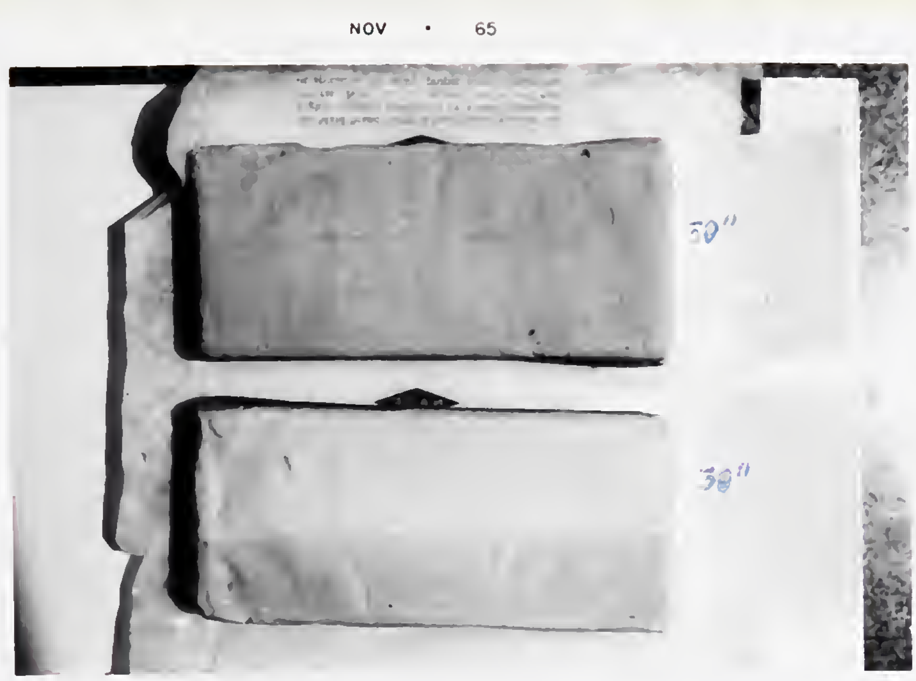

Fig. 2. Butter in parchment wrap for exposure test.
Note side (a) and side (b).

DISCUSSION

During oxidation changes occur in the chemical as well as the biological properties of fats. In our experiments, it was demonstrated that with increasing exposure of butter to fluorescent light, the peroxide and carbonyl values changed greatly and this seemed to be closely paralleled by organoleptic changes.

It appears that the artificial lighting that is used in display cases plays an important part in butter oxidation. In some instances, it was observed that where the carbonyl determination gave very low values, the butter samples did not taste oxidized even if the peroxide values were comparatively high. It has been established that the major components of oxidized flavour in dairy products are carbonyl compounds (39, 40, 76, 78, 143). However, our observations demonstrate that the carbonyls responsible for off-flavour are highly specific. This observation was made by other investigators of oxidized flavour (3, 6, 23, 25, 28, 36).

According to Thome and Mattson (147), butterfat has a relatively long induction period. The results of our tests seem to verify this statement since the butter samples had an average induction period of 2 weeks, at which time large increases in

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peroxide values occurred. The same authors stated that the length of the induction period and the rate of oxidation during the active period, that is, the period when peroxides are formed in large amounts, are affected by the contents of conjugated C18 fatty acids and linolenic acid. The variations in the rate of oxidation of the different fats during the active period are attributed to variations in the contents of dienoic and trienoic acids. Dugan (32) noted that longer incubation periods at lower temperatures tended to bring out odours and flavours which will make a fat unfit for many uses, even though it is not rancid either in terms of peroxide value or organoleptic evaluation. The results of our experiments seem in agreement with this finding. This is especially observed with the samples exposed in the laboratory.

The parchment covered samples that were exposed at a distance of 10 inches from the light seemed to have a well-defined induction period. The interior samples had the same well-defined induction period although they did not develop high peroxide values. According to Patton (106), dairy products follow the common hydroperoxide mechanism of lipid oxidation, that is, they have an induction period during which the amount of peroxides formed increases only slowly, the end of this period is indicated by a sudden increase in peroxide value.

The parchment covered samples exposed at a distance of 20 inches from the light also had a distinct induction period. The higher total carbonyl contents were accompanied by relatively low peroxide values. The interior samples also had high carbonyl values at the time of maximum carbonyl formation at the surface. The organoleptic tests indicated that the fat at this point was already highly oxidized. Henick et al. (67), in their study of corn and soybean oils autoxidized at 98.7°C, observed that the content of unsaturated carbonyls exceeded that of the saturated ones, which only increased markedly at a later stage of rancidity. Berry and McKerrigan (6) believed that this was due to the fact that initially carbonyl compounds formed from linolenic and linoleic acyl groups were unsaturated; breakdown of the primary carbonyl compounds by scission then led to unsaturated or saturated short chain carbonyls, and because these compounds have powerful off-flavours they will apparently result in the appearance of organoleptic rancidity.

It appears that butterfat does not develop oxidized flavours as fast as butter does. This seems to agree with the findings of Patton (106) who argued that with butter which represents an aqueous system of phospholipids dispersed in fat, both fat and phospholipids are susceptible to oxidation, the latter being more easily oxidized. It appears that there is a

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difference in the susceptibility of phospholipids to oxidation depending upon whether they are present in fat or water. The butter samples had lower unsaturated carbonyl values than butterfat. This seems to indicate that the presence of phospholipids has an effect on the formation of unsaturated carbonyls, it may be that the phospholipids inhibit the formation of carbonyls or they may promote the rapid breakdown of carbonyls so that they are not detected in the test. The latter argument seems favored by various studies (120,140, 145, 147, 149, 150). A similar pattern seems to be followed by the results listed in tables 7 and 8. Swanson and Sommer (140) have shown that the development of oxidized flavour is primarily due to the partial oxidation of phospholipids. This was verified by the results of a study made ny Riel and Sommer (120).

While the peroxide value is used as a measure of oxidation of fats, it is not usually a good criterion for flavour score of fat (32). Evans (36) observed in his experiments with soybean oil that the peroxide value is not consistent with the flavour score, especially when the oil was subjected to different conditions of oxidation. This seems to be the case also with the results of the experiments presented in Tables 1 to 8. Many of the butter samples with peroxide values of less than 1 tasted

highly oxidized and butter samples with higher peroxide values did not give any oxidized flavour. In some cases, samples having the same or nearly the same peroxide values had different flavour scores. Caution is needed therefore, in the interpretation of relationship of peroxide value and flavour score as was mentioned by Dugan (32).

Results of our experiments show there are cases when butter samples with very high carbonyl values tasted only slightly oxidized. This seems to support the findings of Badings (3), who suggested that an autoxidizing fat is in a dynamic state and therefore any chemical or organoleptic tests reflect only the situation of the oxidizing fat at the particular time of sampling. However, several samples with very low carbonyl values gave highly oxidized flavour. According to Keeney and Doan (76) oxidation products responsible for off-flavour are perceptible in far smaller concentration than can be detected chemically. This was also shown by Patton (107), who reported that deca-2,4-dienal can be detected organoleptically at a concentration of one part per billion. It appears that in this particular condition, organoleptic evaluation would be more sensitive than chemical tests since it would be difficult to measure the compounds responsible for off-flavour at this very low level.

The bleaching of the butter exposed with parchment cover is illustrated by a series of photographs. They were taken weekly for four weeks to show the changes in colour. It appears that both the distance from and the length of time to exposure to the light have an effect on the butter. A study of the photographs reveals a gradual bleaching of the samples from the first week to the fourth week, when the surface colour was almost completely bleached. There were, however, fine points that cannot readily be observed in the photographs such as for example, the darker blotches where the butter forms crevices which did not receive as much light as the rest of the surface, and the slightly darker colour where the parchment paper did not adhere evenly to the butter surface.

It can be concluded from the results presented in this thesis, that light induced oxidation of butter in retail stores is a serious problem. It is possible that the quality of butter available to the consumer is greatly affected by oxidative deterioration.

Several remedies can be suggested, one is the replacement of the commonly used cool white lamps with lamps having less of the harmful short wave length radiation. This is not likely to afford complete protection and does not seem to offer a practical solution to the problem. The second possibility is the shielding of the butter from the light by packaging in aluminum foil or cardboard containers. Although the cost of the packaging material will be somewhat higher, this will give full protection, and is already in practical use in some locations. It appears that this approach deserves careful consideration by the butter industry, as it will result in greatly improved butter quality and increased consumer satisfaction.

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